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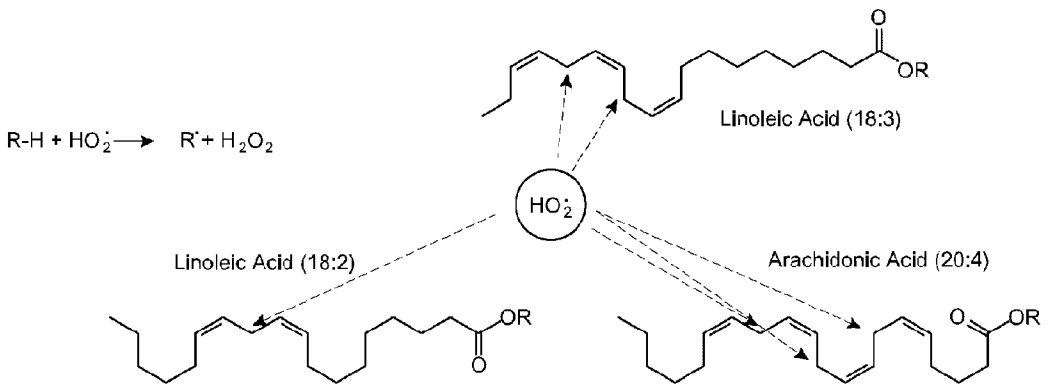
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(54) Title: NEURODEGENERATIVE DISORDERS AND MUSCLE DISEASES IMPLICATING PUFAS



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Some aspects of the invention provide for a method of treating Alzheimer's Disease, Mild Cognitive Impairment, Frontotemperal Dementia, Amyotrophic Lateral Sclerosis and/or Multiple Sclerosis using polyunsaturated fatty acids which are modified in certain positions to attenuate oxidative damage by Reactive Oxygen Species (ROS) and/or suppress the rate of formation of reactive products and toxic compounds.

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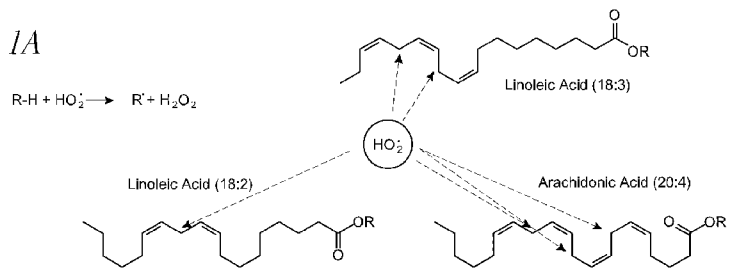
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FIG. 1A



(57) Abstract: Some aspects of the invention provide for a method of treating Alzheimer's Disease, Mild Cognitive Impairment, Frontotemperal Dementia, Amyotrophic Lateral Sclerosis and/or Multiple Sclerosis using polyunsaturated fatty acids which are modified in certain positions to attenuate oxidative damage by Reactive Oxygen Species (ROS) and/or suppress the rate of formation of reactive products and toxic compounds.

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NEURODEGENERATIVE DISORDERS AND MUSCLE DISEASES IMPLICATING PUFAS

BACKGROUND

Cross-Reference to Related Applications

[0001] This application claims the benefit of priority to U.S. Application No. 61/479,270, filed April 26, 2011 and U.S. Application No. 61/479,269, filed April 26, 2011.

Field

[0002] Isotopically modified polyunsaturated fatty acids ("PUFAs") and other modified PUFAs for treating certain diseases, particularly Alzheimer's Disease, Mild Cognitive Impairment, Frontotemperal Dementia, Amyotrophic Lateral Sclerosis and Multiple Sclerosis.

Description of the Related Art

[0003] Oxidative damage is implicated in a wide variety of diseases such as mitochondrial diseases, neurodegenerative diseases, neurodegenerative muscle diseases, retinal diseases, energy processing disorders, kidney diseases, hepatic diseases, lipidemias, cardiac diseases, inflammation, and genetic disorders. Specifically, such diseases include but are not limited to Alzheimer's Disease (AD), Mild Cognitive Impairment (MCI), and Frontotemperal Dementia (FD).

[0004] While the number of diseases associated with oxidative stress are numerous and diverse, it is well established that oxidative stress is caused by disturbances to the normal redox state within cells. An imbalance between routine production and detoxification of reactive oxygen species ("ROS") such as peroxides and free radicals can result in oxidative damage to cellular structures and machinery. Under normal conditions, a potentially important source of ROSs in aerobic organisms is the leakage of activated oxygen from mitochondria during normal oxidative respiration. Additionally, it is known that macrophages and enzymatic reactions also contribute to the generation of ROSs within cells. Because cells and their internal organelles are lipid membrane-bound, ROSs can readily contact membrane constituents and cause lipid oxidation. Ultimately, such oxidative damage can be relayed to other biomolecules within the cell, such as DNA and proteins, through direct and indirect contact with activated oxygen, oxidized membrane constituents, or other oxidized cellular components. Thus, one can readily envision how oxidative damage may propagate throughout a cell give the mobility of internal constituents and the interconnectedness of cellular pathways.

[0005] Lipid-forming fatty acids are well-known as one of the major components of living cells. As such, they participate in numerous metabolic pathways, and play an important role in a variety of pathologies. Polyunsaturated Fatty Acids ("PUFAs") are an important sub-class of fatty acids. An essential nutrient is a food component that directly, or *via* conversion, serves an essential biological function and which is not produced endogenously or in large enough amounts to cover the requirements. For homeothermic animals, the two rigorously essential PUFAs are linoleic (*cis*,*cis*-9,12-Octadecadienoic acid; (9*Z*,12*Z*)-9,12-Octadecadienoic acid; "LA"; 18:2;n-6) and alpha-linolenic (*cis*,*cis*,*cis*-9,12,15-Octadecatrienoic acid; (9*Z*,12*Z*,15*Z*)-9,12,15-Octadecatrienoic acid; "ALA"; 18:3;n-3) acids, formerly known as vitamin F (Cunnane SC. Progress in Lipid Research **2003**; 42:544–568). LA, by further enzymatic desaturation and elongation, is converted into higher n-6 PUFAs such as arachidonic (AA; 20:4;n-6) acid; whereas ALA gives rise to a higher n-3 series, including, but not limited to, eicosapentaenoic acid (EPA; 20:5;n-3) and docosahexaenoic (DHA; 22:6;n-3) acid (Goyens PL. et al. *Am. J. Clin. Nutr.* **2006**; *84*:44-53). Because of the essential nature of certain PUFAs or PUFA precursors, there are many known instances of their deficiency and these are often linked to medical conditions. Furthermore, many PUFA supplements are available over-the-counter, with proven efficiency against certain ailments (See, for example, U.S. Patent No.: 7,271,315 and U.S. Patent No.: 7,381,558).

[0006] PUFAs endow mitochondrial membranes with appropriate fluidity necessary for optimal oxidative phosphorylation performance. PUFAs also play an important role in initiation and propagation of the oxidative stress. PUFAs react with ROS through a chain reaction that amplifies an original event (Sun M, Salomon RG, *J. Am. Chem. Soc.* **2004**; *126*:5699-5708). However, non-enzymatic formation of high levels of lipid hydroperoxides is known to result in several detrimental changes. Indeed, Coenzyme Q10 has been linked to increased PUFA toxicity via PUFA peroxidation and the toxicity of the resulting products (Do TQ et al, *PNAS USA* **1996**; *93*:7534-7539). Such oxidized products negatively affect the fluidity and permeability of their membranes; they lead to oxidation of membrane proteins; and they can be converted into a large number of highly reactive carbonyl compounds. The latter include reactive species such as acrolein, malonic dialdehyde, glyoxal, methylglyoxal, etc. (Negre-Salvayre A, et al. *Brit. J. Pharmacol.* **2008**; *153*:6-20). But the most prominent products of PUFA oxidation are alpha, beta-unsaturated aldehydes such as 4-hydroxynon-2-enal (4-HNE; formed from n-6 PUFAs like LA or AA), 4-hydroxyhex-2-enal (4-HHE; formed from n-3 PUFAs like ALA or DHA), and corresponding ketoaldehydes (Esterfbauer H, et al. *Free Rad. Biol. Med.* **1991**; *11*:81-128; Long EK, Picklo MJ. *Free Rad. Biol. Med.* **2010**; *49*:1-8). These

reactive carbonyls cross-link (bio)molecules through Michael addition or Schiff base formation pathways, and have been implicated in a large number of pathological processes (such as those introduced above), age-related and oxidative stress-related conditions, and aging. Importantly, in some cases, PUFAs appear to oxidize at specific sites because methylene groups of 1,4-diene systems (the bis-allylic position) are substantially less stable to ROS, and to enzymes such as cyclogenases and lipoxygenases, than allylic methylenes.

[0007] We have now discovered that oxidation resistant PUFAs, PUFA mimetics, PUFA pro-drugs and/or fats containing oxidation resistant PUFAs and PUFA mimetics that are useful for treating and/or inhibiting neurodegenerative disorders.

SUMMARY

[0008] Some embodiments provide a method of treating or inhibiting the progression of neurodegenerative disorders, comprising administering an effective amount of a polyunsaturated substance to an Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia patient in need of treatment, wherein the polyunsaturated substance is chemically modified such that one or more bonds are stabilized against oxidation, wherein the polyunsaturated substance or a polyunsaturated metabolite thereof comprising said one or more stabilized bonds is incorporated into the patient's body following administration. Other embodiments provide a method of treating or inhibiting the progression of neuromuscular disease, comprising administering an effective amount of a polyunsaturated substance to an Amyotrophic Lateral Sclerosis or Multiple Sclerosis patient in need of treatment, wherein the polyunsaturated substance is chemically modified such that one or more bonds are stabilized against oxidation, wherein the polyunsaturated substance or a polyunsaturated metabolite thereof comprising said one or more stabilized bonds is incorporated into the patient's body following administration.

[0008a] Various embodiments of the claimed invention relate to a polyunsaturated substance for use in the preparation of a medicine for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester in the medicine is about 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

[0008b] Various embodiments of the claimed invention relate to a polyunsaturated substance for use for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at

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one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester in the medicine is at least 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

[0008c] Various embodiments of the claimed invention relate to use of a polyunsaturated substance for use for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester in the medicine is about 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

[0008d] Various embodiments of the claimed invention relate to use of a polyunsaturated substance for use in the preparation of a medicine for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester in the medicine is at least 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

[0008e] Various embodiments of the claimed invention relate to use of a polyunsaturated substance for use in the preparation of a medicine for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester in the medicine is about 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

[0008f] Various embodiments of the claimed invention relate to use of a polyunsaturated substance for use in the preparation of a medicine for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a

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polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester in the medicine is about 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

[0008g] Various embodiments of the claimed invention relate to use of a polyunsaturated substance for use for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester is about 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

[0009] In some embodiments, the polyunsaturated substance is a nutrition element. In other embodiments, the nutrition element is a fatty acid, a fatty acid mimetic, and/or a fatty acid pro-drug. In other embodiments, the nutrition element is a triglyceride, a diglyceride, and/or a monoglyceride comprising a fatty acid, a fatty acid mimetic, and/or a fatty acid pro-drug. In some embodiments, the fatty acid, fatty acid mimetic, or fatty acid pro-drug is stabilized at one or more bis-allylic positions. In other embodiments, the stabilization comprises at least one ^{13}C atom or at least one deuterium atom at a bis-allylic position. In some embodiments, the stabilization comprises at least two deuterium atoms at one or more bis-allylic position. In other embodiments, the stabilization utilizes an amount of isotopes that is above the

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naturally-occurring abundance level. In some embodiments, the stabilization utilizes an amount of isotopes that is significantly above the naturally-occurring abundance level of the isotope.

[0010] In some embodiments, the fatty acid, fatty acid mimetic, or fatty acid pro-drug has an isotopic purity of from about 20%-99%. In other embodiments, the isotopically stabilized fatty acids, fatty acid mimetics, or fatty acid pro-drugs are administered to a patient along with non-stabilized fatty acids, fatty acid mimetics, or fatty acid pro-drugs. In some embodiments, the isotopically stabilized fatty acids, fatty acid mimetics, or fatty acid pro-drugs comprise between about 1% and 100%, between about 5% and 75%, between about 10% and 30%, or about 20% or more of the total amount of fatty acids, fatty acid mimetics, or fatty acid pro-drugs administered to the patient. In some embodiments, the patient ingests the fatty acid, fatty acid mimetic, or fatty acid pro-drug. In some embodiments, a cell or tissue of the patient maintains a sufficient concentration of the fatty acid, fatty acid mimetic, fatty acid pro-drug, triglyceride, diglyceride, and/or monoglyceride to prevent autooxidation of the naturally occurring polyunsaturated fatty acid, mimetic, or ester pro-drug. In some embodiments, the stabilization utilizes an amount of isotope that is significantly above the naturally-occurring abundance level of said isotope.

[0011] In some embodiments, the method utilizes a fatty acid, fatty acid mimetic, or fatty acid pro-drug that is an omega-3 fatty acid and/or an omega-6 fatty acid. In other embodiments, the fatty acid selected from the group consisting of 11,11-D2-linolenic acid, 14,14-D2-linolenic acid, 11,11,14,14-D4-linolenic acid, 11,11-D2-linoleic acid, 14,14-D2-linoleic acid, 11,11,14,14-D4-linoleic acid, 11-D-linolenic acid, 14-D-linolenic acid, 11,14-D2-linolenic acid, 11-D-linoleic acid, 14-D-linoleic acid, and 11,14-D2-linoleic acid. In other embodiments, the fatty acids are further stabilized at a pro-bis-allylic position. In some embodiments, the fatty acid is alpha linolenic acid, linoleic acid, gamma linolenic acid, dihomo gamma linolenic acid, arachidonic acid, and/or docosatetraenoic acid. In some embodiments, the fatty acid is incorporated into the mitochondrial membrane. In other embodiments, the fatty acid pro-drug is an ester. In some embodiments, the ester is a triglyceride, diglyceride, or monoglyceride.

[0012] Some embodiments further comprise co-administering an antioxidant. In some embodiments, the antioxidant is Coenzyme Q, idebenone, mitoquinone, or mitoquinol. In other embodiments, the antioxidant is a mitochondrially-targeted antioxidant. In some embodiments, the antioxidant is a vitamin, vitamin mimetic, or vitamin pro-drug. In other embodiments, the antioxidant is a vitamin E, vitamin E mimetic, vitamin E pro-drug, vitamin C, vitamin C mimetic, and/or vitamin C pro-drug.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **Figures 1A and 1B.** (1A) ROS-driven oxidation of PUFAs; (1B) formation of toxic carbonyl compounds.

[0014] **Figures 2A and 2B.** ^{1}H- and ^{13}C-NMR analysis of deuterated PUFAs described in Examples 1-4.

[0015] **Figure 3.** Sensitivity of coq null mutants to treatment with linolenic acid is abrogated by isotope-reinforcement. Yeast coq3, coq7 and coq9 null mutants were prepared in the W303 yeast genetic background (WT). Yeast strains were grown in YPD medium (1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose) and harvested while in log phase growth (OD_{600nm}=0.1-1.0). Cells were washed twice with sterile water and resuspended in phosphate buffer (0.10 M sodium phosphate, pH 6.2, 0.2% dextrose) to an OD_{600nm}=0.2. Samples were removed and 1:5 serial dilutions starting at 0.20 OD/ml were plated on YPD plate medium, to provide a zero time untreated control (shown in top left panel). The designated fatty acids were added to 200 μM final concentration to 20 ml of yeast in phosphate buffer. At 2 h, 4 h, and 16 h samples were removed, 1:5 serial dilutions prepared, and spotted onto YPD plate medium. Pictures were taken after 2 days of growth at 30°C. This panel is representative of two independent assays, performed on different days.

[0016] **Figure 4.** Yeast coq mutants treated with isotope-reinforced D4-linolenic acid are resistant to PUFA-mediated cell killing. The fatty acid sensitive assay was performed as described in Figure 3, except that 100 μl aliquots were removed at 1, 2, and 4 h and, following dilution, spread onto YPD plates. Pictures were taken after 2 to 2.5 days, and the number of colonies counted. Yeast strains include Wild type (circles), atp2 (triangles), or coq3 (squares); Fatty acid treatments include oleic C18:1 (solid line), linolenic, C18:3, n-3 (dashed line) or 11,11,14,14-D4-linolenic, C18:3, n-3, (dotted line).

[0017] **Figure 5.** Separation and detection of fatty acid methyl ester (FAME) standards by GC-MS. FAMEs were prepared as described (Moss CW, Lambert MA, Merwin WH. *Appl. Microbiol.* **1974**; 1, 80-85), and the indicated amounts of free fatty acids and 200 μg of C17:0 (an internal standard) were subjected to methylation and extraction. Samples analyses were performed on an Agilent 6890-6975 GC-MS with a DB-wax column (0.25 mm X 30 m X 0.25-m film thickness) (Agilent, catalog 122-7031).

[0018] **Figure 6.** Uptake of exogenously supplied fatty acids by yeast. WT (W303) yeast were harvested at log phase and incubated in the presence of 200 μM of the designated fatty acid for either 0 or 4 h. Yeast cells were harvested, washed twice with sterile water and then subjected to alkaline methanolysis and saponification, and lipid extraction as described

(Moss CW, Lambert MA, Merwin WH. *Appl. Microbiol.* **1974**; 1, 80-85; **(Shaw, 1953** Shaw, W. H. C.; Jefferies, J. P. Determination of ergosterol in yeast. *Anal Chem* **25:**1130; 1953**)**. Each designated fatty acid is given as μg per OD_{600nm} yeast, and was corrected for the recovery of the C17:0 internal standard.

[0019] **Figure 7.** Kinetics of O_2 consumption accompanied the oxidation of 0.71 M LA (plots 1 and 2) and 0.71 M D2-LA (plot 3) in chlorobenzene initiated by 40 mM AMVN at 37° C. Plot 2 – 0.23 mM HPMC was added to 0.71 M LA.

[0020] **Figure 8.** Dependence of the rate of oxidation of the mixture of LA and D2-LA in chlorobenzene solution on mixture composition. Conditions: [LA] + [11,11-d_2-LA] = 0.775 M; [AMVN] = 0.0217 M; 37° C. $R_{IN} = (1.10 \pm 0.08) \times 10^{-7}$ M/scc.

[0021] **Figure 9.** Isotope reinforcement at the bis-allylic position of polyunsaturated fatty acids attenuates lipid autoxidation. Wild-type, yeast Q-less *coq3,* or respiratory deficient *cor1* null mutants were incubated in the presence of 200 μM of LA and D2-LA at different ratios of PUFAs. Serial dilutions (1:5) starting at 0.2OD/ml were spotted on YPD solid plate medium. A zero-time untreated control is shown on the top left. Growth at 30° C.

[0022] **Figure 10.** Isotope reinforcement at the bis-allylic position of polyunsaturated fatty acids attenuates lipid autoxidation. Wild-type, yeast Q-less *coq3,* or respiratory deficient *cor1* null mutants were incubated in the presence of 200 μM of ALA and D4-LA at different ratios of PUFAs. Serial dilutions (1:5) starting at 0.2OD/ml were spotted on YPD solid plate medium. Growth at 30° C.

[0023] **Figure 11.** Chromatograms of the yeast extracts subjected to GC-MS analyses. The different traces represent the 0 and 4 h incubations, respectively. The peak area of Each FAME (C18:1, C18:3 and D4-linolenic) was divided by the peak area of the C17:0 standard, quantified with a calibration curve. The endogenous 16:0 and 16:1 changes very little, while the exogenously added fatty acids increased significantly.

[0024] **Figure 12.** Survival of H- and D-PUFA treated MVEC cells after acute intoxication by paraquat. For all cell types tested, D-PUFA had protective effects compared to controls, similar to that shown for MVEC cells.

[0025] **Figure 13.** Animal dosage studies of 1:1 D2-LA/D4-ALA indicating tissue enrichment with deuterium.

[0026] **Figure 14.** Animal dosage studies of 1:1 D2-LA/D4-ALA comparing any changes in fat distribution.

[0027] **Figure 15.** Animal dosage studies of 1:1 D2-LA/ALA indicating tissue enrichment with deuterium.

[0028] **Figure 16.** Control liver fat profile after 90-day animal dosage study.

[0029] **Figure 17.** Animal dosage studies of 1:1 D2-LA/D4-ALA indicating liver fat profile and enrichment with deuterium.

[0030] **Figure 18.** Liver fat profile after 90-day animal dosage study with D2-LA.

[0031] **Figure 19.** Animal dosage studies of 1:1 D2-LA/D4-ALA indicating brain fat profile and enrichment with deuterium.

[0032] **Figure 20.** Animal dosage studies of 1:1 D2-LA/ALA indicating brain fat profile and enrichment with deuterium.

[0033] **Figure 21.** Control brain fat profile after 90-day animal dosage study.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0034] As used herein, abbreviations are defined as follows:

αLnn	Alpha-linolenic acid
4-HHE or HHE	4-Hydroxyhex-2-enal
4-HNE	4-Hydroxynon-2-enal
AA	Arachidonic (AA; 20:4;n-6) acid
Ab	Amyloid beta
AcOH	Acetic acid
AD	Alzheimer's Disease
ADRDA	Alzheimer's Disease and Related Disorders Association
AGE	Advanced glycation end products
ALA	Alpha-linolenic acid
ALS	Amyotrophic Lateral Sclerosis
AMVN	2,2′-Azobis(2,4-dimethylvaleronitrile)
a-syn	Alpha-synucleon
D	Deuterated
D1	Mono-deuterated
D2	Di-deuterated
D2-LA	Di-deuterated linoleic acid
D3	Tri-deuterated
D4	Tetra-deuterated
D5	Penta-deuterated
D6	Hexa-deuterated
DHA	Docosahexaenoic (22:6; n-3) acid
DMF	Dimethylformamide
EPA	Eicosapentaenoic (20:5; n-3) acid
EtOAc	Ethyl acetate
EtOH	Ethanol
FAME	Fatty acid methyl ester
FD	Frontotemperal Dementia
HPMC	6-Hydroxy-2,2,5,7,8-pentamethylbenzochroman
H-PUFA	Non-deuterated polyunsaturated fatty acid
IP	Intraperitoneal
IR	Infrared
IsoP	15-F-isoprostane

KIE	Kinetic isotope effect
LA	Linolcic acid
LDL	Low-density lipoprotein
MCI	Mild Cognitive Impairment
MDA	Malondialdehyde
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MS	Multiple Sclerosis
MVEC	Microvascular endothelium
NINCDS	Neurological and Communicative Disorders and Stroke
ONE	Omega 6 peroxidation products
PUFA(s)	Polyunsaturated fatty acid(s)
R_{IN}	Rate of initiation
ROS	Reactive oxygen species
R_{OX}	Rate of oxidation
sALS	Sporadic Amyotrophic Lateral Sclerosis
SNCA	Alpha-syn gcnc
SNOMED	Systematized Nomenclature of Medicine
SOD	Superoxide dismutase
TDMS	Toxicology Data Management System
TH	Tyrosine hydroxylase
THF	Tetrahydrofuran
TLC	Thin layer chromatography
V-SMOW	Vienna standard mean ocean water
WT	Wild type
YPD	Medium containing 1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose

Alzheimer's Disease, Mild Cognitive Impairment, and Frontotemperal Dementia

[0035] Amyloid plaques and neurofibrillary tangles are the neuropathological hallmarks of AD, although whether they are the cause or the product of the disease is still debatable. For additional information, see Cooper JL. *Drugs & Aging* **2003**; *20*:399-418. Oxidative stress, and a related inflammation, is implicated in the AD process. *See* Pattern et al., *Journal of Alzheimer's Disease* (2010); 20, S357-S367. The direct evidence supporting increased oxidative stress in AD is: (1) increased ROS-stimulating Fe, Al, and Hg in the AD subject's brain; (2) increased PUFA peroxidation and decreased PUFAs in the AD subject's brain, and increased 4-HNE in the AD subject's ventricular fluid; (3) increased protein and DNA oxidation in the AD subject's brain; (4) diminished energy metabolism and decreased cytochrome c oxidase in the AD subject's brain; (5) advanced glycation end products (AGE), MDA, carbonyls, peroxynitrite, heme oxygenase-1 and SOD-1 in neurofibrillary tangles and AGE, heme oxygenase-1, SOD-1 in senile plaques; and (6) studies showing that amyloid beta peptide is capable of generating ROS (Markesbery WR. *Free Rad. Biol. Med.* **1997**; *23*:134-147). Moreover, mitochondrial dysfunction is implicated in many neurodegenerative diseases

and oxidative stress is known to induce dysfunction. *See* Schon et al., *Journal of Alzheimer's Disease* (2010); 20, S281-S292; Zhu et al., *Journal of Alzheimer's Disease* (2010); 20, S253; Filippo et al., *Journal of Alzheimer's Disease* (2010); 20, S369-S379; Morais et al., *Journal of Alzheimer's Disease* (2010); 20, S255-S263; Coskun et al., *Journal of Alzheimer's Disease* (2010); 20, S293-S310; and Swerdlow et al., *Journal of Alzheimer's Disease* (2010); 20, S265-S279.

[0036] The abnormalities of lipid metabolism play a prominent role in AD. All proteins involved in Amyloid precursor protein processing and Ab peptide production are integral membrane proteins. Moreover, the Ab producing c-secretase cleavage takes place in the middle of the membrane, so the lipid environment of the cleavage enzymes influences Ab production and AD pathogenesis (Hartmann T. et al, *J. Neurochem.* **2007**;*103*:159-170).

[0037] Lipid peroxidation is marked by high levels of malondialdehyde, isoprostanes, and high levels of protein modification by HNE and acrolein (Sayre LM, et al. *Chem. Res. Toxicol.* **2008**; *21*:172-188; Butterfield DA, et al. *Biochim. Biophys. Acta* **2010**; *1801*:924-929). Dietary PUFAs are the principal risk factor for the development of late-onset sporadic AD. The degree of saturation of PUFAs and the position of the first double bond are the most critical factors determining the risk of AD, with unsaturated fats and n-3 double bonds conferring protection and an overabundance of saturated fats or n-6 double bonds increasing the risk. DHA and AA are particularly relevant to AD (Luzon-Toro B, et al. *Neurol. Psychiatr. Brain Res.* **2004**; *11*:149-160). DHA is the major component of excitable membranes, promotes maturation in infants and is a potent neuroprotective agent in the adult brain, with a potential role in the prevention of AD. AA is an important provider of eicosanoids, acting as a second messenger in many neurotransmitter systems. The interaction of dietary PUFAs and apolipoprotein E isoforms may determine the risk and rate of sustained autoperoxidation within cellular membranes and the efficacy of membrane repair.

[0038] ROS-initiated PUFA peroxidation, also known as PUFA autoxidation, can be mitigated by the quenching of ROS with antioxidants. A large number of antioxidants exist, comprising hydrophobic antioxidants such as vitamin E; hydrophilic antioxidants such as vitamin C; antioxidant enzymes such as Superoxide dismutases; and other types of compounds. However, reactive carbonyl products of PUFA peroxidation are not of a free radical nature and cannot be neutralized by antioxidants. Antioxidants are known to prevent lipid peroxidation protected primary rat hippocampal neurons against apoptosis induced by oxidative insults. However, the antioxidants did not protect these neuronal cells against HNE-induced apoptosis (Kruman I. et al, *J. Neurosci.*, **1997**, *17*:5089-5100). Increased levels of free NHE were detected

in multiple brain regions in AD compared with age-matched control subjects. These increases reached statistical significance in the amygdala and hippocampus and parahippocampal gyrus, regions showing the most pronounced histopathological alterations in AD, confirming the importance of HNE in the pathogenesis of neuron degeneration in AD (Markesbery W.R. et al, *Neurobiol. of Aging* **1998**; *19*:33-36).

[0039] Increased stability of reactive carbonyls compared to ROS permits for their diffusion away from the formation site. They can damage other components elsewhere in the cell, for example cross-linking proteins and reacting with nucleic acid bases. Such modified DNA bases may possess complementary properties different from the standard Watson-Crick base pairing, causing detrimental mutations and other damage. For example, there is a two-fold increase in DNA damage in certain tissues of patients with MCI and AD (Migliore L et al, *Neurobiol. Aging* **2005**; *26*:587-595). Similar observations were reported for FD (Gerst J.L. et al, *Dement Geriatr Cogn Disord* **1999**; *10*:85-87).

[0040] It has been reported that lipid peroxidation is present in the brain of MCI patients. Several studies established oxidative damage as an early event in the pathogenesis of AD and such damage can serve as a therapeutic target to slow the progression or perhaps the onset of the disease. (Markesbery WR. *Arch. Neurol.* **2007**; *64*:954-956). MCI can also be characterized by elevated levels of conjugates formed by lipid peroxidation products such as MDA, HNE, acrolein and isoprostanes (Butterfield DA, et al. *Biochim. Biophys. Acta* **2010**; *1801*:924-929).

[0041] Identifying subjects with Alzheimer's disease or susceptible to Alzheimer's disease are known in the art. For instance, subjects may be identified using criteria set forth by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS)-Alzheimer's Disease and Related Disorders Association (ADRDA). The criteria are related to memory, language, perceptual skills, attention, constructive abilities, orientation, problem solving and functional abilities. Similar diagnostic tests may be used to identify MCI patients.

Amyotrophic Lateral Sclerosis

[0042] Amyotrophic Lateral Sclerosis (ALS), a motor neuron disease, is a late-onset progressive neurodegenerative disorder (loss of upper and lower motor neurons), that culminates in muscle wasting and death from respiratory failure (Boillee S et al, *Neuron* **2006**;*52*:39-59). Familial ALS (fALS; about 2% of all cases) is caused by misfolding of mutated Cu/Zn SOD-1 (Kabashi E et al, *Ann. Neurol.* **2007**;*62*:553-559). There are more than 100 mutations in SOD that are associated with the fALS (Barnham KJ et al, *Nature Rev. Drug Discov.* **2004**;*3*:205-214). The first step is the 'monomerisation' of SOD, which then leads to the aggregation of SOD

monomers, which form aberrant S-S bonds between themselves (Kabashi E et al, *Ann. Neurol.* **2007**;*62*:553-559), yielding toxic conglomerates (either because they mis-fold, or because they become a source of ROS, or both (Barnham KJ et al, *Nature Rev. Drug Discov.* **2004**;*3*:205-214)). Studies on a G93A-SOD1 model linked fALS-associated SOD1 mutations with its loss of redox sensor function in NADPH oxidase-dependent ROS production, leading to microglial neurotoxic inflammatory responses, mediated by an uncontrolled ROS generation (Liu Y et al, *JBC* **2009**;*284*:3691-3699). Sporadic ALS (sALS) is more common (90% cases). Another hallmark feature of ALS is the neuronal cytoplasmic and intranuclear aggregation of RNA-binding protein TDP-43 (Dennis JS. et al, *Neuroscience* **2009**;*158*:745-750).

[0043] The etiology of ALS cases remains unknown, but it is widely recognized that ALS is associated with oxidative stress and inflammation. Protein oxidation is up 85% in sALS patients (Coyle JT et al, *Science* **1993**;*262*:689-695), and increased lipid peroxidation and 4-hydroxynonenal (HNE) and 4-hydroxyhexenal (HHE) formation have been reported for ALS cases, both familial and sporadic (Simpson EP et al, *Neurology* **2004**;*62*:1758-1765; Shibata N et al, *Brain Res.* **2004**;*1019*:170-177). This has been observed in central nervous system (CNS) tissue, spinal fluid, and serum. Inhibition of COX-2 has been reported to reduce spinal neurodegeneration and prolong the survival of ALS transgenic mice (Minghetti L. *J Neuropathol Exp Neurol* **2004**;*63*:901-910), suggesting a role for PUFA oxidation products in the etiology of ALS. The source of the oxidative stress in ALS is not clear but may derive from several processes including excitotoxicity, mitochondrial dysfunction, iron accumulation or immune activation (Simpson EP et al, *Neurology* **2004**;*62*:1758-1765). There is evidence that mitochondria play an important role in fALS and sALS, being both a trigger and a target for oxidative stress in ALS (Bacman SR et al, *Molec. Neurobiol.* **2006**;*33*:113-131). *See also* Martin, *Journal of Alzheimer's Disease* (2010); 20, S335-S356; Shi et al., *Journal of Alzheimer's Disease* (2010); 20, S311-S324; Glicksman, Expert. Opin. Drug. Disc. (2011) 6:11; 1127-1138

[0044] Despite the association of oxidative stress with ALS, clinical trials using antioxidant therapies have so far failed in ALS and other CNS diseases (Barber SC et al, *Biochim. Biophys. Acta* **2006**;*1762*:1051-1067). These trials may have failed for several reasons: (a) antioxidants are usually present in cells at high (virtually saturated) concentrations, and further supplementation leads to only marginal increases (Zimniak P *Ageing Res. Rev.* **2008**;*7*:281-300). The stochastic nature of ROS-inflicted damage is therefore not sensitive to antioxidant therapies; (b) ROS themselves are important in cell signaling and other processes, including the requirement for low levels of ROS for hormetic (adaptive) upregulation of

protective mechanisms; (c) some antioxidants (such as vitamin E) can become potent oxidants themselves, capable of initiating PUFA autoxidation (Bowry VW et al, *JACS* **1993** ;*115* :6029-6044); and (d) antioxidants are ineffective in neutralizing the carbonyl compounds like HNE and HHE, because HNE and HHE, once formed, react in different ways compared to the free radical mechanism and so cannot be quenched by typical antioxidants.

[0045] Lipid peroxidation, one of the first and major outcomes of oxidative stress, is particularly pronounced in CNS disease, as the CNS is enriched in polyunsaturated fatty acids (PUFAs; second highest concentration after the adipose tissue). PUFA peroxidation occurs at the bis-allylic methylene groups (between double bonds) and leads to the subsequent liberation of α,β-unsaturated carbonyl derivatives such as acrolein, 4-HNE, ONE, 4-HHE, crotonaldehyde, etc. Recent research suggests that the strongest detrimental effect on the etiology of oxidative stress-related diseases, including neurological disorders, is exercised specifically by electrophilic toxicity of reactive carbonyl compounds (Zimniak P *Ageing Res. Rev.* **2008**;*7*:281-300). These carbonyl compounds (see above) can cause nerve terminal damage by forming adducts with presynaptic proteins. Therefore, the endogenous generation of acrolein, HNE, HHE and the like in oxidatively stressed neurons of certain brain regions is mechanistically related to the synaptotoxicity associated with neurodegenerative conditions. In addition, acrolein, acrylamide, crotonaldehyde, HNE, HHE etc are members of a large class of structurally related chemicals known as the type-2 alkenes, which are toxic to nerve terminals. Regional synaptotoxicity, which develops during the early stages of many neurodegenerative diseases, is mediated by endogenous generation of reactive carbonyl compounds from oxidized PUFAs. Moreover, the onset and progression of this neuropathogenic process is accelerated by environmental exposure to other type-2 alkenes. Toxic carbonyls formed from both omega-3 and omega-6 PUFA have been shown to play a role in etiology of ALS. HNE and ONE (omega-6 peroxidation products) levels are elevated in both fALS and sALS (Simpson EP et al, *Neurology* **2004**;*62*:1758-1765; Adibhatla RM, et. al. *Antioxidants Redox Signaling* **2010** ;*12* :125-169). HHE and crotonaldehyde (both omega-3 peroxidation products) form protein conjugates in the spinal cord during ALS (Shibata N. et al. *Brain Res.* **2004**;*1019*:170-177; Shibata N et al, *Neuropathol.* **2007**;*27*:49-61). Analysis of ALS-associated protein damage on a G93A-SOD1 mouse model of the disease reveals that several spinal cord proteins are substantially HNE-modified, including the heat shock protein Hsp70 (Perluigi M et al, *FRBM* **2005**;*38*:960-968), supporting the role of oxidative stress as a major mechanism in the pathogenesis of ALS. Another indication of ALS-associated PUFA oxidation is an increased level of 15-F-isoprostane (IsoP), a product of ROS-mediated PUFA peroxidation (Mitsumoto H

et al, *ALS* **2008**;*9*:177-183). DNA damage by PUFA peroxidation, through NHE and ONE conjugation with DNA bases, leads to activation of the p53 signaling pathway, which is involved in ALS neurodegeneration (Adibhatla RM, et. al. *Antioxidants Redox Signaling* **2010**;*12* :125-169).

Multiple Sclerosis

[0046] PUFA peroxidation and reactive carbonyl compounds play an important role in MS. Extensive oxidative damage to proteins, lipids and nucleotides in active demyelinating MS lesions, predominantly in reactive astrocytes and myelin-laden macrophages has been reported, including a substantial presence of reactive carbonyl products such as HNE (van Horssen J. et al, *Free Rad. Biol. Med.* **2008**;*45*:1729-1737). It was also established that LDL can enter the parenchyma of early MS lesions as a result of blood-brain barrier damage, thus representing another source of reactive carbonyls such as malonic dialdehyde and 4-HNE in MS plaques (Newcombe J. et al, *Neuropathol. and Applied Neurobiol.* **1994**;*20*:152-162).

[0047] Identifying a subject having or at risk for developing ALS and MS may be determined using diagnostic methods known in the art. For example, one or a combination of tests may be used such as upper and lower motor neuron signs in a single limb; electromyography (EMG); nerve conduction velocity (NCV) measurement to rule out peripheral neuropathy and myopathy; magnetic resonance imaging (MRI); and/or blood and urine testing to eliminate a possibility of other diseases.

[0048] Some aspects of this invention arise from: (1) an understanding that while essential PUFAs are vital for proper functioning of lipid membranes, and in particular of the mitochondrial membranes, their inherent drawback, i.e., the propensity to be oxidized by ROS with detrimental outcome, is implicated in AD, MCI, and FD; (2) antioxidants cannot prevent PUFA peroxidation due to stochastic nature of the process and the stability of PUFA peroxidation products (reactive carbonyls) to antioxidant treatment, and (3) the ROS-driven damage of oxidation-prone sites within PUFAs may be overcome by using an approach that makes them less amenable to such oxidations, without compromising any of their beneficial physical properties. Some aspects of this invention describe the use of the isotope effect to achieve this, only at sites in essential PUFAs and PUFA precursors that matter most for oxidation, while other aspects contemplate other sites in addition to those that matter most for oxidation.

[0049] Moreover, isotopically labeled embodiments should have minimal or non-existent effects on important biological processes. For example, the natural abundance of isotopes present in biological substrates implies that low levels of isotopically labeled

compounds should have negligible effects on biological processes. Additionally, hydrogen atoms are incorporated into biological substrates from water, and is it known that the consumption of D_2O, or heavy water, does not pose a health threat to humans. *See*, e.g., "Physiological effect of heavy water." *Elements and isotopes : formation, transformation, distribution.* Dordrecht: Kluwer Acad. Publ. (2003) pp. 111–112 (indicating that a 70 kg person might drink 4.8 liters of heavy water without serious consequences). Moreover, many isotopically labeled compounds are approved by the U.S. Food & Drug Administration for diagnostic and treatment purposes.

[0050] It will be appreciated by those skilful in the art that the same effect as an isotope effect can be achieved by protecting oxidation-prone positions within PUFAs using other chemical approaches. Certain PUFA mimetics, while possessing structural similarity with natural PUFAs, will nevertheless be stable to ROS-driven oxidation due to structural reinforcement.

Compositions:

[0051] In some embodiments, an isotopically modified polyunsaturated fatty acid or a mimetic refers to a compound having structural similarity to a naturally occurring PUFA that is stabilized chemically or by reinforcement with one or more isotopes, for example ^{13}C and/or deuterium. Generally, if deuterium is used for reinforcement, one or both hydrogens on a methylene group may be reinforced.

[0052] Some aspects of this invention provide compounds that are analogues of essential PUFAs with either one, several, or all bis-allylic positions substituted with heavy isotopes. In some embodiments, the CH_2 groups, which will become the bis-allylic position in a PUFA upon enzymatic conversion, are substituted with one or two heavy isotopes. Such compounds are useful for the prevention or treatment of diseases in which PUFA oxidation is a factor or can contribute to disease progression.

[0053] The bis-allylic position generally refers to the position of the polyunsaturated fatty acid or mimetic thereof that corresponds to the methylene groups of 1,4-diene systems. The pro-bis-allylic position refers to the methylene group that becomes the bis-allylic position upon enzymatic desaturation.

[0054] In some embodiments, the chemical identity of PUFAs, i.e., the chemical structure without regard to the isotope substitutions or substitutions that mimic isotope substitutions, remains the same upon ingestion. For instance, the chemical identity of essential PUFAs, that is, PUFAs that mammals such as humans do not generally synthesize, may remain identical upon ingestion. In some cases, however, PUFAs may be further extended/desaturated

in mammals, thus changing their chemical identity upon ingestion. Similarly with mimetics, the chemical identity may remain unchanged or may be subject to similar extension/desaturation. In some embodiments, PUFAs that are extended, and optionally desaturated, upon ingestion and further metabolism may be referred to as higher homologs.

[0055] In some embodiments, naturally-occurring abundance level refers to the level of isotopes, for example ^{13}C and/or deuterium that may be incorporated into PUFAs that would be relative to the natural abundance of the isotope in nature. For example, ^{13}C has a natural abundance of roughly 1% ^{13}C atoms in total carbon atoms. Thus, the relative percentage of carbon having greater than the natural abundance of ^{13}C in PUFAs may have greater than the natural abundance level of roughly 1% of its total carbon atoms reinforced with ^{13}C, such as 2%, but preferably about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 65%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of ^{13}C with respect to one or more carbon atoms in each PUFA molecule. In other embodiments, the percentage of total carbon atoms reinforced with ^{13}C is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 65%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

[0056] Regarding hydrogen, in some embodiments, deuterium has a natural abundance of roughly 0.0156% of all naturally occurring hydrogen in the oceans on earth. Thus, a PUFA having greater that the natural abundance of deuterium may have greater than this level or greater than the natural abundance level of roughly 0.0156% of its hydrogen atoms reinforced with deuterium, such as 0.02%, but preferably about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 65%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of deuterium with respect to one or more hydrogen atoms in each PUFA molecule. In other embodiments, the percentage of total hydrogen atoms reinforced with deuterium is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 65%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

[0057] In some aspects, a composition of PUFAs contains both isotopically modified PUFAs and isotopically unmodified PUFAs. The isotopic purity is a comparison between a) the relative number of molecules of isotopically modified PUFAs, and b) the total molecules of both isotopically modified PUFAs and PUFAs with no heavy atoms. In some embodiments, the isotopic purity refers to PUFAs that are otherwise the same except for the heavy atoms.

[0058] In some embodiments, isotopic purity refers to the percentage of molecules of an isotopically modified PUFAs in the composition relative to the total number of molecules of the isotopically modified PUFAs plus PUFAs with no heavy atoms. For example, the isotopic purity may be about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 65%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the molecules of isotopically modified PUFAs

relative to the total number of molecules of both the isotopically modified PUFAs plus PUFAs with no heavy atoms. In other embodiments, the isotopic purity is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 65%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, isotopic purity of the PUFAs may be from about 10%-100%, 10%-95%, 10%-90%, 10%-85%, 10%-80%, 10%-75%, 10%-70%, 10%-65%, 10%-60%, 10%-55%, 10%-50%, 10%-45%, 10%-40%, 10%-35%, 10%-30%, 10%-25%, or 10%-20% of the total number of molecules of the PUFAs in the composition. In other embodiments, isotopic purity of the PUFAs may be from about 15%-100%, 15%-95%, 15%-90%, 15%-85%, 15%-80%, 15%-75%, 15%-70%, 15%-65%, 15%-60%, 15%-55%, 15%-50%, 15%-45%, 15%-40%, 15%-35%, 15%-30%, 15%-25%, or 15%-20% of the total number of molecules of the PUFAs in the composition. In some embodiments, isotopic purity of the PUFAs may be from about 20%-100%, 20%-95%, 20%-90%, 20%-85%, 20%-80%, 20%-75%, 20%-70%, 20%-65%, 20%-60%, 20%-55%, 20%-50%, 20%-45%, 20%-40%, 20%-35%, 20%-30%, or 20%-25% of the total number of molecules of the PUFAs in the composition. Two molecules of an isotopically modified PUFA out of a total of 100 total molecules of isotopically modified PUFAs plus PUFAs with no heavy atoms will have 2% isotopic purity, regardless of the number of heavy atoms the two isotopically modified molecules contain.

[0059] In some aspects, an isotopically modified PUFA molecule may contain one deuterium atom, such as when one of the two hydrogens in a methylene group is replaced by deuterium, and thus may be referred to as a "D1" PUFA. Similarly, an isotopically modified PUFA molecule may contain two deuterium atoms, such as when the two hydrogens in a methylene group are both replaced by deuterium, and thus may be referred to as a "D2" PUFA. Similarly, an isotopically modified PUFA molecule may contain three deuterium atoms and may be referred to as a "D3" PUFA. Similarly, an isotopically modified PUFA molecule may contain four deuterium atoms and may be referred to as a "D4" PUFA. In some embodiments, an isotopically modified PUFA molecule may contain five deuterium atoms or six deuterium atoms and may be referred to as a "D5" or "D6" PUFA, respectively.

[0060] The number of heavy atoms in a molecule, or the isotopic load, may vary. For example, a molecule with a relatively low isotopic load may contain about 1, 2, 3, 4, 5, or 6 deuterium atoms. A molecule with a moderate isotopic load may contain about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 deuterium atoms. In a molecule with a very high load, every hydrogen may be replaced with a deuterium. Thus, the isotopic load refers to the percentage of heavy atoms in each PUFA molecule. For example, the isotopic load may be about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 65%, 60%, 65%, 70%, 75%, 80%, 85%, 90%,

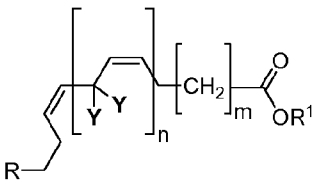
95%, or 100% of the number of the same type of atoms in comparison to a PUFA with no heavy atoms of the same type (e.g. hydrogen would be the "same type" as deuterium). In some embodiments, the isotopic load is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 65%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. Unintended side effects are expected to be reduced where there is high isotopic purity in a PUFA composition but low isotopic load in a given molecule. For example, the metabolic pathways will likely be less affected by using a PUFA composition with high isotopic purity but low isotopic load.

[0061] One will readily appreciate that when one of the two hydrogens of a methylene group is replaced with a deuterium atom, the resultant compound may possess a stereocenter. In some embodiments, it may be desirable to use racemic compounds. In other embodiments, it may be desirable to use enantiomerically pure compounds. In additional embodiments, it may be desirable to use diastereomerically pure compounds. In some embodiments, it may be desirable to use mixtures of compounds having enantiomeric excesses and/or diastereomeric excesses of about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 65%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In other embodiments, the enantiomeric excesses and/or diastereomeric excesses is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 65%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, it may be preferable to utilize stereochemically pure enantiomers and/or diastereomers of embodiments – such as when contact with chiral molecules is being targeted for attenuating oxidative damage. However, in many circumstances, non-chiral molecules are being targeted for attenuating oxidative damage. In such circumstances, embodiments may be utilized without concern for their stereochemical purity. Moreover, in some embodiments, mixtures of enantiomers and diastereomers may be used even when the compounds are targeting chiral molecules for attenuating oxidative damage.

[0062] In some aspects, isotopically modified PUFAs impart an amount of heavy atoms in a particular tissue. Thus, in some aspects, the amount of heavy molecules will be a particular percentage of the same type of molecules in a tissue. For example, the number of heavy molecules may be about 1%-100% of the total amount of the same type of molecules. In some aspects, 10-50% the molecules are substituted with the same type of heavy molecules.

[0063] In some embodiments, a compound with the same chemical bonding structure as an essential PUFA but with a different isotopic composition at particular positions will have significantly and usefully different chemical properties from the unsubstituted compound. The particular positions with respect to oxidation, including oxidation by ROS, comprise bis-allylic positions of essential polyunsaturated fatty acids and their derivatives, as shown in **Figure 1**.

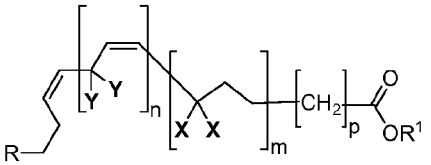
The essential PUFAs isotopically reinforced at bis-allylic positions shown below will be more stable to the oxidation. Accordingly, some aspects of the invention provide for particular methods of using compounds of Formula (1) or salts thereof, whereas the sites can be further reinforced with carbon-13. R^1 = alkyl, H, or cation; m = 1-10; n = 1-5, where at each bis-allylic position, one or both Y atoms are deuterium atoms, for example,



(1) R = H, C_3H_7; R^1 = H, alkyl, or cation; Y = H or D

11,11-Dideutero-cis,cis-9,12-Octadecadienoic acid (11,11-Dideutero-(9Z,12Z)-9,12-Octadecadienoic acid; D2-LA); and 11,11,14,14-Tetradeutero-cis,cis,cis-9,12,15-Octadecatrienoic acid (11,11,14,14-Tetradeutero-(9Z,12Z,15Z)-9,12,15-Octadecatrienoic acid; D4-ALA). In some embodiments, said positions, in addition to deuteration, can be further reinforced by carbon-13, each at levels of isotope abundance above the naturally-occurring abundance level. All other carbon-hydrogen bonds in the PUFA molecule may optionally contain deuterium and/or carbon-13 at, or above, the natural abundance level.

[0064] Essential PUFAs are biochemically converted into higher homologues by desaturation and elongation. Therefore, some sites which are not bis-allylic in the precursor PUFAs will become bis-allylic upon biochemical transformation. Such sites then become sensitive to oxidation, including oxidation by ROS. In a further embodiment, such pro-bis-allylic sites, in addition to existing bis-allylic sites are reinforced by isotope substitution as shown below. Accordingly, this aspect of the invention provides for the use of compounds of Formula (2) or salt thereof, where at each bis-allylic position, and at each pro-bis-allylic position, one or more of X or Y atoms may be deuterium atoms. R1 = alkyl, cation, or H; m = 1-10; n = 1-5; p = 1-10.

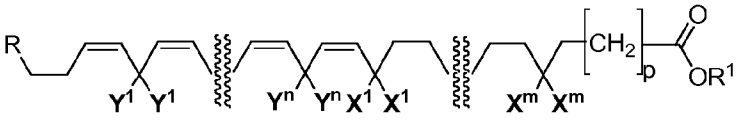


(2) R = H, C_3H_7; R^1 = H, alkyl, or cation; Y = H or D; X = H or D

[0065] Said positions, in addition to deuteration, can be further reinforced by carbon-13, each at levels of isotope abundance above the naturally-occurring abundance level. All other carbon-hydrogen bonds in the PUFA molecule may contain optionally deuterium and/or carbon-13 at or above the natural abundance level.

[0066] Oxidation of PUFAs at different bis-allylic sites gives rise to different sets of oxidation products. For example, 4-HNE is formed from n-6 PUFAs whereas 4-HHE is formed

from n-3 PUFAs (Negre-Salvayre A, et al. *Brit. J. Pharmacol.* **2008**; *153*:6-20). The products of such oxidation possess different regulatory, toxic, signaling, etc. properties. It is therefore desirable to control the relative extent of such oxidations. Accordingly, some aspects of the invention provide for the use of compounds of Formula (3), or salt thereof, differentially reinforced with heavy stable isotopes at selected bis-allylic or pro-bis-allylic positions, to control the relative yield of oxidation at different sites, as shown below, such that any of the pairs of Y^1-Y^n and/or X^1-X^m at the bis-allylic or pro-bis-allylic positions of PUFAs may contain deuterium atoms. R1 = alkyl, cation, or H; m = 1-10; n = 1-6; p = 1-10



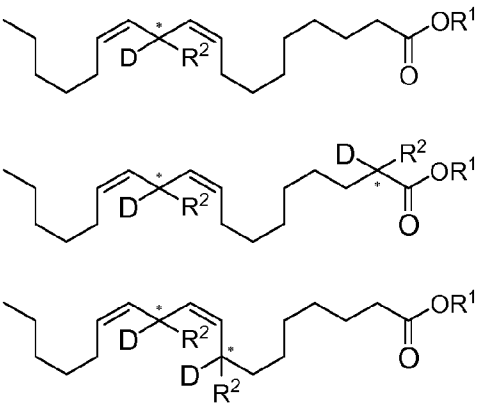
(3)

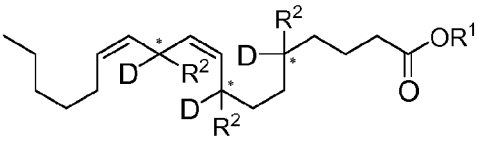
R = H, C_3H_7; R^1 = H, alkyl, or cation; Y = H or D; X = H or D

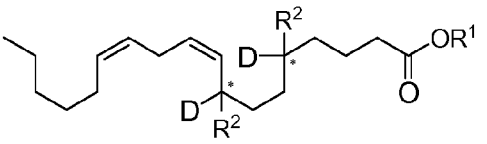
[0067] Said positions, in addition to deuteration, can be further reinforced by carbon-13. All other carbon-hydrogen bonds in the PUFA molecule may contain deuterium at, or above the natural abundance level. It will be appreciated that the break lines in the structure shown above represents a PUFA with a varying number of double bonds, a varying number of total carbons, and a varying combination of isotope reinforced bis-allylic and pro-bis-allylic sites.

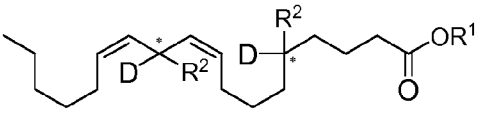
[0068] Exact structures of compounds illustrated above are shown below that provide for both isotope reinforced n-3 (omega-3) and n-6 (omega-6) essential polyunsaturated fatty acids, and the PUFAs made from them biochemically by desaturation/elongation. Any one of these compounds may be used to slow oxidation. In the following compounds, the PUFAs are isotopically reinforced at oxidation sensitive sites and/or sites that may become oxidation sensitive upon biochemical desaturation/elongation. R^1 may be H, alkyl, or cation; R^2 may be H or D; * represents either ^{12}C or ^{13}C.

[0069] D-Linoleic acids include:

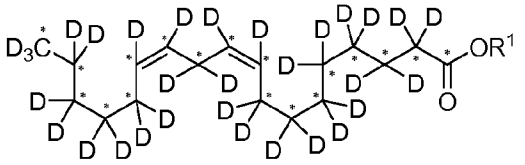




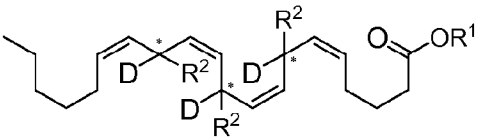


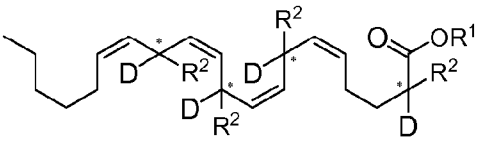


[0070] The per-deuterated linoleic acid below may be produced by microbiological methods, for example by growing in media containing deuterium and/or carbon-13.

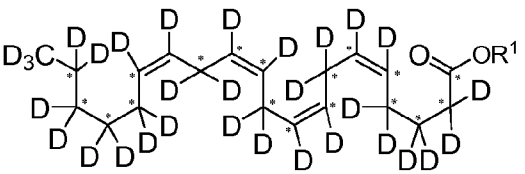


[0071] D-Arachidonic acids include:

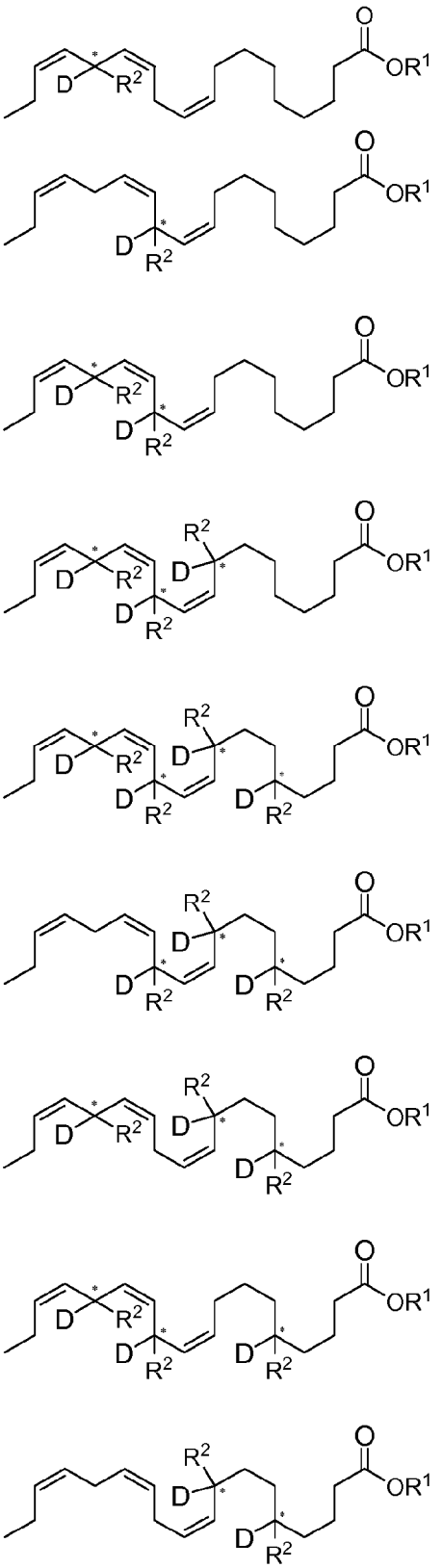


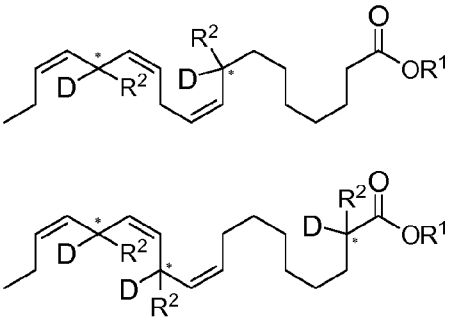


[0072] The per-deuterated arachidonic acid below may be produced by microbiological methods, such as by growing in media containing deuterium and/or carbon-13.

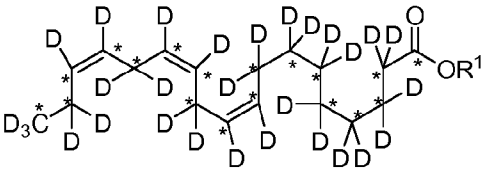


[0073] D-Linolenic acids include:





[0074] Per-deuterated linolenic acid below may be produced by microbiological methods, such as growing in media containing deuterium and/or carbon-13.



[0075] In some aspects of the invention, any PUFAs, whether essential or not, that are capable of being taken up from diet and used in the body, can be utilized. In the case of essential or non-essential PUFAs or precursors, the supplemented stabilized materials can compete with other dietary uptake and bio-manufacture to reduce the available disease-causing species concentrations.

[0076] In some aspects of the invention, the PUFAs isotopically reinforced at oxidation sensitive positions as described by way of the structures above are heavy isotope enriched at said positions as compared to the natural abundance of the appropriate isotope, deuterium and/or carbon-13.

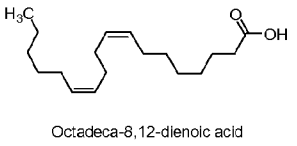
[0077] In some embodiments, the disclosed compounds are enriched to 99% isotope purity or more. In some embodiments, the heavy isotope enrichment at said positions is between 50%-99% deuterium and/or carbon-13.

[0078] In some embodiments, the modified fatty acids, when dosed via diet as drugs or supplements, may be dosed as pro-drugs, including, but not limited to, non-toxic and pharmaceutically suitable esters of the parent fatty acid or mimetic, such as an ethyl ester or glyceryl ester. This ester assists in tolerance of the drug in the gut, assists in digestion, and relies on the high levels of esterases in the intestines to de-esterify the ester pro-drugs into the active acid form of the drug which adsorbs. Hence, in some embodiments, the invention encompasses the pro-drug esters of the modified fatty acids herein. Examples of this type of drug in the market, nutrition, and clinical trials literature, including Glaxo's Lovaza, (mixtures of omega 3 fatty acid esters, EPA, DHA, and alpha-linolenic acid), Abbott's Omacor (omega-3-fatty acid esters), and most fish oil supplements (DHA and EPA esters). In some aspects,

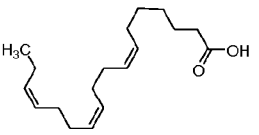
incorporation of the ester pro-drugs into tissues or cells refers to the incorporation of the modified parent PUFA as it would be used as a bodily constituent.

[0079] In some embodiments, stabilized compositions mimic natural occurring fatty acids without changing their elemental composition. For example, the substituent may retain the chemical valence shell. Some embodiments include naturally occurring fatty acids, mimetics, and their ester pro-drugs, that are modified chemically to be effective at preventing specific disease mechanisms, but are modified in a way (such as isotopic substitution) that does not change the elemental composition of the material. For example, deuterium is a form of the same element hydrogen. In some aspects, these compounds maintain elemental composition and are stabilized against oxidation. Some compounds that are stabilized against oxidation are stabilized at oxidation sensitive loci. Some compounds are stabilized against oxidation via heavy isotope substitution, then at bis-allylic carbon hydrogen bonds, etc.

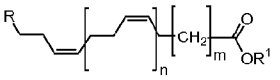
[0080] In a further embodiment, oxidation-prone bis-allylic sites of PUFAs can be protected against hydrogen abstraction by moving bis-allylic hydrogen-activating double bonds further apart, thus eliminating the bis-allylic positions while retaining certain PUFA fluidity as shown below. These PUFA mimetics have no bis-allylic positions.



Octadeca-8,12-dienoic acid

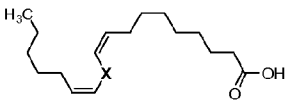


Octadeca-7,11,15-trienoic acid

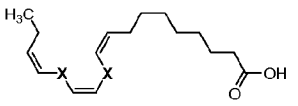


R = H, C_3H_7; R^1 = H; alkyl; n = 1-4;m = 1-12

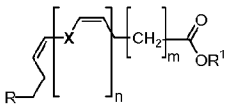
[0081] In a further embodiment, oxidation-prone bis-allylic sites of PUFAs can be protected against hydrogen abstraction by using heteroatoms with valence II, thus eliminating the bis-allylic hydrogens as shown below. These PUFA mimetics also have no bis-allylic hydrogens.



X = S: 10-Hept-1-enylsulfanyl-dec-9-encic acid
X = O: 10-Hept-1-enyloxy-dec-9-enoic acid

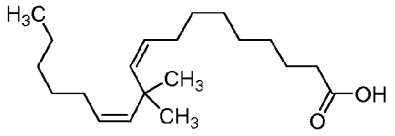


X = S: 10-(2-But-1-enylsulfanyl-vinylsulfanyl)-dec-9-enoic acid
X = O:10-(2-But-1-enyloxy-vinyloxy)-dec-9-encic acid

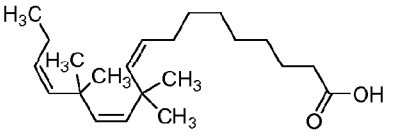


R = H, C_3H_7; R^1 = H; alkyl; X = O; S; n = 1-5; m = 1-12

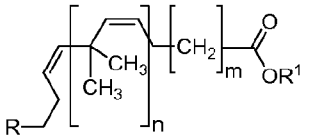
[0082] In a further embodiment, PUFA mimetics, i.e. compounds structurally similar to natural PUFAs but unable to get oxidized because of the structural differences, can be employed for the above mentioned purposes. Oxidation-prone bis-allylic sites of PUFAs can be protected against hydrogen abstraction by di-methylation or halogenation as shown below. The hydrogen atoms on the methyl groups may optionally be halogens, such as fluorine, or deuterium. These PUFA mimetics are dimethylated at bis-allylic sites.



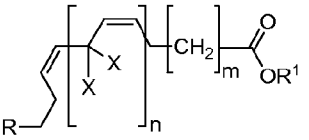
11,11-Dimethyl-octadeca-9,12-dienoic acid



11,11,14,14-Tetramethyl-octadeca-9,12,15-trienoic acid

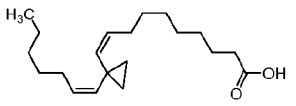


R = H, C_3H_7; R^1 = H; alkyl; n = 1-5; m = 1-12

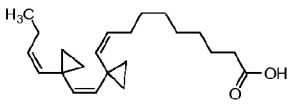


R = H, C_3H_7; R^1 = H; alkyl; n = 1-5; m = 1-12; X = F, Cl., Br, or I

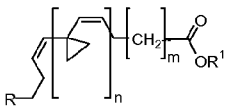
[0083] In a further embodiment, oxidation-prone bis-allylic sites of PUFAs can be protected against hydrogen abstraction by alkylation as shown below. These PUFA mimetics are dialkylated at bis-allylic sites.



10-(1-Hept-1-enyl-cyclopropyl)-dec-9-enoic acid

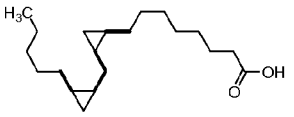


10-{1-[2-(1-But-1-enyl-cyclopropyl)-vinyl]-cyclopropyl}-dec-9-enoic acid

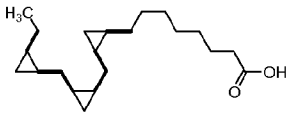


R = H, C_3H_7; R^1 = H; alkyl; n = 1-5; m = 1-12

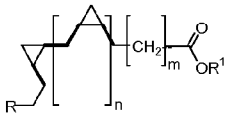
[0084] In a further embodiment, cyclopropyl groups can be used instead of double bonds, thus rendering the acids certain fluidity while eliminating the bis-allylic sites as shown below. These PUFA mimetics have cyclopropyl groups instead of double bonds.



8-[2-(2-Pentyl-cyclopropylmethyl)-cyclopropyl]-octanoic acid

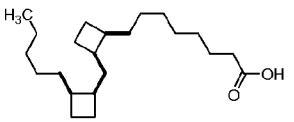


8-{2-[2-(2-Ethyl-cyclopropylmethyl)-cyclopropylmethyl]-cyclopropyl}-octanoic acid

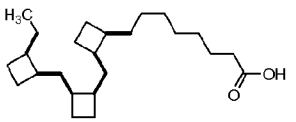


R = H, C_3H_7; R^1 = H; alkyl; n = 1-5; m = 1-12

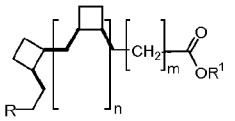
[0085] In a further embodiment, 1,2-substituted cyclobutyl groups in appropriate conformation can be used instead of double bonds, thus rendering the acids certain fluidity while eliminating the bis-allylic sites as shown below. These PUFA mimetics have 1,2-cyclobutyl groups instead of double bonds.



8-[2-(2-Pentyl-cyclobutylmethyl)-cyclobutyl]-octanoic acid



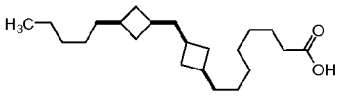
8-{2-[2-(2-Ethyl-cyclobutylmethyl)-cyclobutylmethyl]-cyclobutyl}-octanoic acid



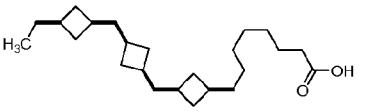
R = H, C_3H_7; R^1 = H; alkyl; n = 1-5; m = 1-12

[0086] In a modification of the previous embodiment of mimetics with 1,2-cyclobutyl groups instead of double bonds, 1,3-substituted cyclobutyl groups in appropriate conformation can be used instead of double bonds, thus rendering the acids certain fluidity while

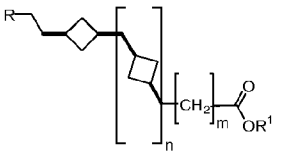
eliminating the bis-allylic sites. The following PUFA mimetics have 1,3-cyclobutyl groups instead of double bonds.



8-[3-(3-Pentyl-cyclobutylmethyl)-cyclobutyl]-octanoic acid



8-{3-[3-(3-Ethyl-cyclobutylmethyl)-cyclobutylmethyl]-cyclobutyl}-octanoic acid



R = H, C_3H_7; R^1 = H; alkyl; n = 1-5; m = 1-12

[0087] It is a well known principle in medicinal chemistry that certain functional groups are isosteric and/or bioisosteric with certain other functional groups. Bioisosteres are substituents or groups with similar physical or chemical properties which produce broadly similar biological properties to a chemical compound. For example, well known isosteres and/or bioisosteres for hydrogen include halogens such as fluorine; isosteres and/or bioisosteres of alkenes include alkynes, phenyl rings, cyclopropyl rings, cyclobutyl rings, cyclopentyl rings, cyclohexyl rings, thioethers, and the like; isosteres and/or bioisosteres of carbonyls include sulfoxides, sulfones, thiocarbonyls, and the like; isosteres and/or bioisosteres of esters include amides, sulfonic acid esters, sulfonamides, sulfinyl acid esters, sulfinylamindes, and the like. Consequently, PUFA mimetics also include compounds having isosteric and/or bioisosteric functional groups.

[0088] It is contemplated that it may be useful to formulate PUFAs and/or PUFA mimetics as a pro-drug for use in the invention. A pro-drug is a pharmacological substance may itself have biological activity, but upon administration the pro-drug is metabolized into a form that also exerts biological activity. Many different types of pro-drugs are known and they can be classified into two major types based upon their cellular sites of metabolism. Type I pro-drugs are those that are metabolized intracellularly, while Type II are those that are metabolized extracellularly. It is well-known that carboxylic acids may be converted to esters and various other functional groups to enhance pharmacokinetics such as absorption, distribution, metabolism, and excretion. Esters are a well-known pro-drug form of carboxylic acids formed by the condensation of an alcohol (or its chemical equivalent) with a carboxylic acid (or its chemical equivalent). In some embodiments, alcohols (or their chemical equivalent) for incorporation into pro-drugs of PUFAs include pharmaceutically acceptable alcohols or

chemicals that upon metabolism yield pharmaceutically acceptable alcohols. Such alcohols include, but are not limited to, propylene glycol, ethanol, isopropanol, 2-(2-ethoxyethoxy)ethanol (Transcutol®, Gattefosse, Westwood, N.J. 07675), benzyl alcohol, glycerol, polyethylene glycol 200, polyethylene glycol 300, or polyethylene glycol 400; polyoxyethylene castor oil derivatives (for example, polyoxyethyleneglyceroltriricinoleate or polyoxyl 35 castor oil (Cremophor®EL, BASF Corp.), polyoxyethyleneglycerol oxystearate (Cremophor®RH 40 (polyethyleneglycol 40 hydrogenated castor oil) or Cremophor®RH 60 (polyethyleneglycol 60 hydrogenated castor oil), BASF Corp.)); saturated polyglycolized glycerides (for example, Gelucire® 35/10, Gelucire® 44/14, Gelucire® 46/07, Gelucire® 50/13 or Gelucire® 53/10, available from Gattefosse, Westwood, N.J. 07675); polyoxyethylene alkyl ethers (for example, cetomacrogol 1000); polyoxyethylene stearates (for example, PEG-6 stearate, PEG-8 stearate, polyoxyl 40 stearate NF, polyoxyethyl 50 stearate NF, PEG-12 stearate, PEG-20 stearate, PEG-100 stearate, PEG-12 distearate, PEG-32 distearate, or PEG-150 distearate); ethyl oleate, isopropyl palmitate, isopropyl myristate; dimethyl isosorbide; N-methylpyrrolidinone; parafin; cholesterol; lecithin; suppository bases; pharmaceutically acceptable waxes (for example, carnauba wax, yellow wax, white wax, microcrystalline wax, or emulsifying wax); pharmaceutically acceptable silicon fluids; soribitan fatty acid esters (including sorbitan laurate, sorbitan oleate, sorbitan palmitate, or sorbitan stearate); pharmaceutically acceptable saturated fats or pharmaceutically acceptable saturated oils (for example, hydrogenated castor oil (glyceryl-tris-12-hydroxystearate), cetyl esters wax (a mixture of primarily C14-C18 saturated esters of C14-C18 saturated fatty acids having a melting range of about 43°-47° C), or glyceryl monostearate).

[0089] In some embodiments, the fatty acid pro-drug is represented by the ester P—B, wherein the radical P is a PUFA and the radical B is a biologically acceptable molecule. Thus, cleavage of the ester P—B affords a PUFA and a biologically acceptable molecule. Such cleavage may be induced by acids, bases, oxidizing agents, and/or reducing agents. Examples of biologically acceptable molecules include, but are not limited to, nutritional materials, peptides, amino acids, proteins, carbohydrates (including monosaccharides, disaccharides, polysaccharides, glycosaminoglycans, and oligosaccharides), nucleotides, nucleosides, lipids (including mono-, di- and tri-substituted glycerols, glycerophospholipids, sphingolipids, and steroids).

[0090] In some embodiments, alcohols (or their chemical equivalent) for incorporation into pro-drugs of PUFAs include alcohols with 1 to 50 carbon atoms ("C_{1-50} alcohols"), C_{1-45} alcohols, C_{1-40} alcohols, C_{1-35} alcohols, C_{1-30} alcohols, C_{1-25} alcohols, C_{1-20}

alcohols, C_{1-15} alcohols, C_{1-10} alcohols, C_{1-6} alcohols (whenever it appears herein, a numerical range such as "1-50" refers to each integer in the given range; *e.g.*, "1 to 50 carbon atoms" means that the alkyl group may consist of 1 carbon atom, 2 carbon atoms, 3 carbon atoms, *etc.*, up to and including 50 carbon atoms, although the present definition also covers the occurrence of the term "alkyl" where no numerical range is designated). Such alcohols may be branched, unbranched, saturated, unsaturated, polyunsaturated and/or include one or more heteroatoms such as nitrogen, oxygen, sulfur, phosphorus, boron, silicone, fluorine, chlorine, bromine, or iodine. Exemplary alcohols include methyl, ethyl, propyl, iso-propyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, hexyl, perfluromethyl, perchloromethyl, perfluoro-tert-butyl, perchloro-tert-butyl, and benzyl alcohols as well as ether alcohols such as polyethylene glycols. In some embodiments, the alcohol contains a charged species. Such species may be anionic or cationic. In some embodiments, the species is a positively charged phosphorus atom. In other embodiments, the positively charged phosphorus atom is a phosphonium cation. In other embodiments the charged species is a primary, secondary, tertiary, or quaternary ammonium cation.

[0091] In some embodiments, alcohols (or their chemical equivalent) for incorporation into pro-drugs of PUFAs include polyalcohols such as diols, triols, tetra-ols, penta-ols, etc. Examples of polyalcohols include ethylene glycol, propylene glycol, 1,3-butylene glycol, polyethylene glycol, methylpropanediol, ethoxydiglycol, hexylene glycol, dipropylene glycol glycerol, and carbohydrates. Esters formed from polyalcohols and PUFAs may be mono-esters, di-esters, tri-esters, etc. In some embodiments, multiply esterified polyalcohols are esterified with the same PUFAs. In other embodiments, multiply esterified polyalcohols are esterified with different PUFAs. In some embodiments, the different PUFAs are stabilized in the same manner. In other embodiments, the different PUFAs are stabilized in different manners (such as deuterium substitution in one PUFA and ^{13}C substitution in another PUFA). In some embodiments, one or more PUFAs is an omega-3 fatty acid and one or more PUFAs is an omega-6 fatty acid.

[0092] It is also contemplated that it may be useful to formulate PUFAs and/or PUFA mimetics and/or PUFA pro-drugs as salts for use in the invention. For example, the use of salt formation as a means of tailoring the properties of pharmaceutical compounds is well known. *See* Stahl et al., Handbook of pharmaceutical salts: Properties, selection and use (2002) Weinheim/Zurich: Wiley-VCH/VHCA; Gould, Salt selection for basic drugs, Int. J. Pharm. (1986), 33:201-217. Salt formation can be used to increase or decrease solubility, to improve stability or toxicity, and to reduce hygroscopicity of a drug product.

[0093] Formulation of PUFAs and/or PUFA mimetics and/or PUFA pro-drugs as salts includes, but is not limited to, the use of basic inorganic salt forming agents, basic organic salt forming agents, and salt forming agents containing both acidic and basic functional groups. Various useful inorganic bases for forming salts include, but are not limited to, alkali metal salts such as salts of lithium, sodium, potassium rubidium, cesium, and francium, and alkaline earth metal salts such as berylium, magnesium, calcium, strontium, barium, and radium, and metals such as aluminum. These inorganic bases may further include counterions such as carbonates, hydrogen carbonates, sulfates, hydrogen sulfates, sulfites, hydrogen sulfites, phosphates, hydrogen phosphates, dihydrogen phosphates, phosphites, hydrogen phosphites, hydroxides, oxides, sulfides, alkoxides such as methoxide, ethoxide, and t-butoxide, and the like. Various useful organic bases for forming salts include, but are not limited to, amino acids, basic amino acids such as arginine, lysine, ornithine and the like, ammonia, alkylamines such as methylamine, ethylamine, dimethylamine, diethylamine, trimethylamine, triethylamine and the like, heterocyclic amines such as pyridine, picoline and the like, alkanolamines such as ethanolamine, diethanolamine, triethanolamine and the like, diethylaminoethanol, dimethylaminoethanol, N-methylglucamine, dicyclohexylamine, N,N′-dibenzylethylenediamine, ethylenediamine, piperazine, choline, trolamine, imidazole, diolamine, betaine, tromethamine, meglumine, chloroprocain, procaine, and the like.

[0094] Salt formulations of PUFAs and/or PUFA mimetics and/or PUFA pro-drugs include, but are not limited to, pharmaceutically acceptable basic inorganic salts, basic organic salts, and/or organic compounds having both acidic and basic functional groups. Pharmaceutically acceptable salts are well known in the art and include many of the above-recited inorganic and organic bases. Pharmaceutically acceptable salts further include salts and salt-forming agents found in drugs approved by the Food and Drug Administration and foreign regulatory agencies. Pharmaceutically acceptable organic cations for incorporation include, but are not limited to, benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, benethamine, clemizole, diethylamine, piperazine, and tromethamine. Pharmaceutically acceptable metallic cations for incorporation include, but are not limited to, aluminum, calcium, lithium, magnesium, potassium, sodium, zinc, barium, and bismuth. Additional salt-forming agents include, but are not limited to, arginine, betaine, carnitine, diethylamine, L-glutamine, 2-(4-imidazolyl)ethylamine, isobutanolamine, lysine, N-methylpiperazine, morpholine, and theobromine.

[0095] Moreover, several lists of pharmaceutically approved counterions exists. See Bighley et al., Salt forms of drugs and absorption. 1996 In: Swarbrick J. et al. eds.

Encyclopaedia of pharmaceutical technology, Vol. 13 New York: Marcel Dekker, Inc. pp 453-499; Gould, P.L., Int. J. Pharm. 1986, 33, 201-217; Berge, J. Pharm. Sci. 1977, 66, 1-19; Heinrich Stahl P., Wermuch C.G. (editors), Handbook of Pharmaceutical Salts, IUPAC, 2002; and Stahl et al., Handbook of pharmaceutical salts: Properties, selection and use (2002) Weinheim/Zurich: Wiley-VCH/VHCA.

[0096] It may be unnecessary to substitute all isotopically unmodified PUFAs, such as non-deuterated PUFAs, with isotopically modified PUFAs such as deuterated PUFAs. In some embodiments, is preferable to have sufficient isotopically modified PUFAs such as D-PUFAs in the membrane to prevent unmodified PUFAs such as H-PUFAs from sustaining a chain reaction of self-oxidation. During self-oxidation, when one PUFA oxidizes, and there is a non-oxidized PUFA in the vicinity, the non-oxidized PUFA can get oxidized by the oxidized PUFA. This may also be referred to as autooxidation. In some instances, if there is a low concentration, for example "dilute" H-PUFAs in the membrane with D-PUFAs, this oxidation cycle may be broken due to the distance separating H-PUFAs. In some embodiments, the concentration of isotopically modified PUFAs is present in a sufficient amount to maintain autooxidation chain reaction. To break the autooxidation chain reaction, for example, 1-60%, 5-50%, or 15-35% of the total molecules of the same type are in the membrane. This may be measured by IRMS (isotope ratio mass spectrometry).

[0097] A further aspect of the invention provides a dietary, supplementary or pharmaceutical composition of the active compounds. In some embodiments, the dietary, supplementary, or pharmaceutical composition may comprise a salt of the active compound.

[0098] Various useful inorganic bases for forming salts include, but are not limited to, alkali metal salts such as salts of lithium, sodium, potassium rubidium, cesium, and francium, and alkaline earth metal salts such as berylium, magnesium, calcium, strontium, barium, and radium, and metals such as aluminum. These inorganic bases may further include counterions such as carbonates, hydrogen carbonates, sulfates, hydrogen sulfates, sulfites, hydrogen sulfites, phosphates, hydrogen phosphates, dihydrogen phosphates, phosphites, hydrogen phosphites, hydroxides, oxides, sulfides, alkoxides such as methoxide, ethoxide, and t-butoxide, and the like.

[0099] Various useful organic bases for forming salts include, but are not limited to, amino acids; basic amino acids such as arginine, lysine, ornithine and the like; ammonia; ammonium hydroxide; alkylamines such as methylamine, ethylamine, dimethylamine, diethylamine, trimethylamine, triethylamine and the like; heterocyclic amines such as pyridine, picoline and the like; alkanolamines such as ethanolamine, diethanolamine, triethanolamine and

the like, diethylaminoethanol, dimethylaminoethanol; N-methylglucamine; dicyclohexylamine; N,N′-dibenzylethylenediamine; ethylenediamine; piperazine; choline; trolamine; imidazole; diolamine; betaine; tromethamine; meglumine; chloroprocain; procaine; and the like.

[0100] Salts of active compounds may include, but are not limited to, pharmaceutically acceptable salts. Pharmaceutically acceptable salts are well known in the art and include many of the above-listed salt-forming agents. Pharmaceutically acceptable salts further include salts and salt-forming agents of the type present in drugs approved by the Food and Drug Administration and foreign regulatory agencies.

[0101] Pharmaceutically acceptable organic cations for incorporation into a salt of an active compound include, but are not limited to, benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, benethamine, clemizole, diethylamine, piperazine, and tromethamine.

[0102] Pharmaceutically acceptable metallic cations for incorporation into a salt of an active compound include, but are not limited to, aluminum, calcium, lithium, magnesium, potassium, sodium, zinc, barium, and bismuth.

[0103] Additional salt-forming agents having potential usefulness as forming salts include, but are not limited to, acetylaminoacetic acid, N-acetyl-L-asparagine, N-acetylcystine, arginine, betaine, carnitine, L-glutamine, 2-(4-imidazolyl)ethylamine, isobutanolamine, lysine, N-methylpiperazine, and morpholine.

[0104] Moreover, several lists of pharmaceutically approved counterions exists. See Bighley et al., Salt forms of drugs and absorption. 1996 In: Swarbrick J. et al. eds. Encyclopaedia of pharmaceutical technology, Vol. 13 New York: Marcel Dekker, Inc. pp 453-499; Gould, P.L., Int. J. Pharm. 1986, 33, 201-217; Berge, J. Pharm. Sci. 1977, 66, 1-19; Heinrich Stahl P., Wermuch C.G. (editors), Handbook of Pharmaceutical Salts, IUPAC, 2002; and, Stahl et al., Handbook of pharmaceutical salts: Properties, selection and use (2002) Weinheim/Zurich: Wiley-VCH/VHCA.

Co-administration

[0105] In some embodiments, the compounds disclosed herein are administered in combination. For example, in some embodiments, two, three, four, and/or five or more stabilized compounds are administered together. In some embodiments, stabilized compounds are administered in approximately similar amounts. In other embodiments, stabilized compounds are administered in differing amounts. For example, any one of two or more compounds in a mixture may represent about 1% to about 99% of a mixture, about 5% to about 95% of a mixture, about 10% to about 90% of a mixture, about 15% to about 85% of a mixture,

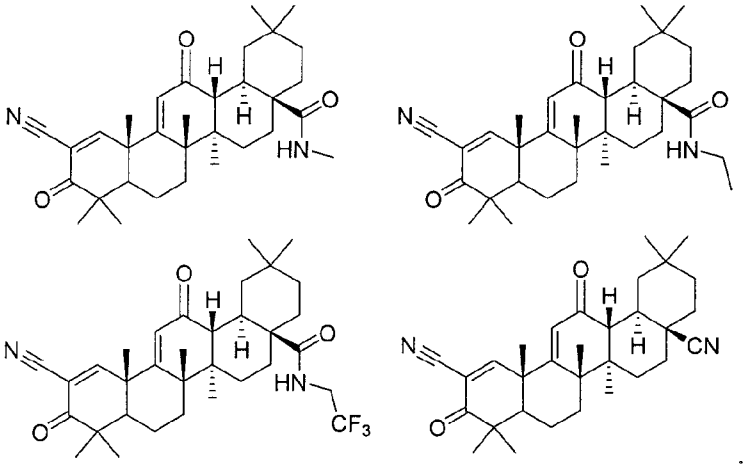
about 20% to about 80% of a mixture, about 25% to about 75% of a mixture, about 30% to about 70% of a mixture, about 35% to about 65% of a mixture, about 40% to about 60% of a mixture, about 40% to about 60% of a mixture, about 45% to about 55% of a mixture, and/or about 50% of a mixture. In other embodiments, any one of two or more compounds in a mixture may represent about: 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 65%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of a mixture.

[0106] Although antioxidants cannot cancel the negative effects of PUFA peroxidation due to the stochastic nature of the process and the stability of PUFA peroxidation products (reactive carbonyls) to antioxidant treatment, co-administration of antioxidants with compositions resistant to oxidation, such as those described herein, may prove beneficial for treating oxidative stress-related disorders. *See* Shrader et al., *Bioorg. Med. Chem. Lett.* (2011), 21(12); 3693-98.

[0107] Certain antioxidants contemplated as useful for co-administration include the following: vitamins, such as vitamin C and vitamin E; glutathione, lipoic acid, uric acid, carotenes, lycopene, lutein, anthocyanins, oxalic acid, phytic acid, tannins, coenzyme Q, melatonin, tocopherols, tocotrienols, polyphenols including resveratrol, flavonoids, selenium, eugenol, idebenone, mitoquinone, mitoquinol, ubiquinone, Szeto-Schiller peptides, and mitochondrial-targeted antioxidants. When not explicitly mentioned, quinone derivatives of the aforementioned antioxidants are also contemplated as useful for co-administration.

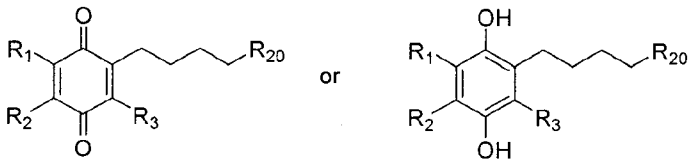
[0108] In some embodiments, stabilized compounds are administered with compounds that upregulate antioxidant genes. In other embodiments, stabilized compounds are administered with compounds that affect signaling pathways, such as the Keap1/Nrf2/ARE signaling pathway, thereby resulting in the production of anti-inflammatory and/or antioxidant proteins, such as heme oxygenase-1 (HO-1). In some embodiments, stabilized compounds are administered with antioxidant inflammation modulators. Antioxidant inflammation modulators suppress pro-oxidant and/or pro-inflammatory transcription factors. In some embodiments, antioxidant inflammation modulators are activators of the transcription factor Nrf2. Nrf2 activation promotes the antioxidant, detoxification, and anti-inflammatory genes upregulation. In other embodiments, antioxidant inflammation modulators suppress NF-κB. In some embodiments, antioxidant inflammation modulators suppress STAT3. In other embodiments, stabilized compounds are administered with compounds that affect histone deacetylase activity. In some embodiments, stabilized compounds are administered with compounds that bind to antioxidant response elements (ARE). In other embodiments, stabilized compounds are administered with bardoxolone methyl (2-cyano-3,12-dioxooleane-1,9(11)-dien-28-oic acid

methyl ester) as the antioxidant inflammation modulator. In some embodiments, the antioxidant inflammation modulator is 2-cyano-3,12-dioxooleane-1,9(11)-dien-28-oic acid, or a pharmaceutically acceptable ester thereof. In other embodiments, the antioxidant inflammation modulator is an amide of 2-cyano-3,12-dioxooleane-1,9(11)-dien-28-oic acid. In some embodiments, the antioxidant inflammation modulator is a triterpenoid. In other embodiments, the antioxidant inflammation modulator is selected from the following compounds:



[0109] Additional antioxidants believed to be useful in co-administration therapies include those compounds disclosed in U.S. Patent Nos. 6,331,532; 7,179,928; 7,232,809; 7,888,334; 7,888,335; 7,432,305; 7,470,798; and 7,514,461; and U.S. Patent Application Nos. 20020052342; 20030069208; 20040106579; 20050043553; 20050245487; 20060229278; 20070238709; 20070270381; 20080161267; 20080275005; 20090258841; 20100029706; and 20110046219. These compounds are mitochondrially-targeted compounds and include, but are not limited to:

[0110] Compounds of Formulas I or II



Formula I or Formula II

wherein R_1 and R_2 are independently selected from $-C_1$-C_4 alkyl, -C_1-C_4 haloalkyl, -CN, -F, -Cl, -Br, and –I; R_3 is selected from $-C_1$-C_4 alkyl, $-C_1$-C_4 haloakyl, -CN, -F, -Cl, and –I, and R_{20} is independently selected from $-C_1$-C_{20} alkyl, $-C_1$-C_{20} alkenyl, $-C_1$-C_{20} alkynyl, and $-C_1$-C_{20} containing at least one double bond and at least one triple bond.

[0111] Compounds such as: 3-(6-Hydroxy-2-methyl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-2-yl)-propionic acid methyl ester; 3-(6-Hydroxy-2-methyl-

3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chroman-2-yl)-propionic acid; 2,2,-Dimethyl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-6-ol; 3-(6-Hydroxy-2-methyl-3,4,7,8,9,10-hexahydro-7,10-propano-2H-benzo[h]chromen-2-yl)-propionic acid methyl ester; 2-Methyl-2-[3-(thiazol-2-ylsulfanyl)-propyl]-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-6-ol; [3-(6-Hydroxy-2-methyl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-2-yl)-propyl]-phosphonic acid dimethyl ester; [3-(6-Hydroxy-2-methyl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-2-yl)-propyl]-phosphonic acid; 3-(6-Hydroxy-2-methyl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-2-yl)-propionic acid methyl ester; 4-(6-Hydroxy-2-methyl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-2-yl)-butane-1-sulfonic acid dimethylamide; 2-(3-Hydroxy-propyl)-2-methyl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-6-ol; 2-(3-Chloro-propyl)-2-methyl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-6-ol 2,2-Dimethyl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-6-ol; -(2-Chloro-ethyl)-2-methyl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-6-ol; 2-Methyl-2-thiazol-2-yl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-6-ol; 2,2-Dimethyl-3,4,7,8,9,10-hexahydro-7,10-ethano-2H-benzo[h]chromen-6-ol; 3-(6-Hydroxy-2-methyl-3,4,7,8,9,10-hexahydro-7,10-ethano-2H-benzo[h]chromen-2-yl)-propionic acid; 2-(3-Chloro-propyl)-2-methyl-3,4,7,8,9,10-hexahydro-7,10-ethano-2H-benzo[h]chromen-6-ol; 4-(6-Hydroxy-2,2-dimethyl-3,4,7,8,9,10-hexahydro-7,10-methano-2H-benzo[h]chromen-5-ylmethylene)-2-methyl-5-propyl-2,4-dihydro-pyrazol-3-one.

[0112] Compounds such as: 2,2,7,8-Tetramethyl-5-phenyl-chroman-6-ol; 4-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-yl)-benzoic acid methyl ester; 4-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-yl)-benzoic acid; 2,2,7,8-Tetramethyl-5-pyridin-4-yl-chroman-6-ol; 2,2,7,8-Tetramethyl-5-pyridin-3-yl-chroman-6-ol; 5-(4-Methanesulfonyl-phenyl)-2,2,7,8-tetramethyl- chroman-6-ol; 5-(4-Dimethylamino-phenyl)-2,2,7,8-tetramethyl-ch roman-6-ol; 5-(4-Chloro-phenyl)-2,2,7,8-tetramethyl-chroman-6-ol; 4-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-yl)-benzenesulfonamide; 5-(4-Methoxy-phenyl)-2,2,7,8-tetramethyl-chroman- 6-ol; (6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethyl)-1-hydroxyurea; 2,2,7,8-Tetramethyl-5-(3-nitro-phenyl)-chroman-6-ol; 2,2,7,8-Tetramethyl-5-(4-trifluoromethyl-phenyl)- chroman-6-ol; 5-(4-tert-Butyl-phenyl)-2,2,7,8-tetramethyl-chrom an-6-ol; 2,2,7,8-Tetramethyl-5-(3,4,5-trimethoxy-phenyl)-c hroman-6-ol; 4-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-yl)-benzonitrile; 5-(2,5-Dimethoxy-3,4-dimethyl-phenyl)-2,2,7,8-tet ramethyl-chroman-6-ol; 5-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-yl)-be nzene-1,2,3-triol; 5-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-yl)-2, 3-dimethyl-benzene-1,4-diol; 5-(2-Chloro-phenyl)-2,2,7,8-

tetramethyl-chroman-6 -ol; 5-Furan-2-yl-2,2,7,8-tetramethyl-chroman-6-ol; 5-Allylsulfanylmethyl-2,2,8-trimethyl-7-(3-methyl-butyl)-chroman-6-ol; 5-Cyclopentylsulfanylmethyl-2,2,7,8-tetramethyl-c hroman-6-ol; 5-Hexylsulfanylmethyl-2,2,7,8-tetramethyl-chroman -6-ol; 5-Allylsulfanylmethyl-2,2,7,8-tetramethyl-chroman -6-ol; 5-(4,6-Dimethyl-pyrimidin-2-ysulfanylmethyl)-2,2,7,8-tetramethyl-chroman-6-ol; 1-[3-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-yl-methylsulfanyl)-2-methyl-propionyl]-pyrrolidine-2-carboxylic acid; 4-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-ylmethylene)-5-methyl-2-phenyl-2,4-dihydro-pyrazol-3-one; 4-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-yl-methylene)-3-phenyl-4H-isoxazol-5-one; 4-[4-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-yl-methylene)-3-methyl-5-oxo-4,5-dihydro-pyrazol-1-yl]-benzoic acid; 4-(6-Hydroxy-2,2,7,8-tetramethyl-chroman-5-yl-methylene)-2-methyl-5-propyl-2,4-dihydro-pyrazol-3-one; 5-Hydroxy-3-(6-hydroxy-2,2,7,8-tetramethyl-chroman-5-yl-methylene)-3H-benzofuran-2-one; 2,5,7,8-Tetramethyl-2-thiophen-2-yl-chroman-6-ol; 2-(2,5-Dimethyl-thiophen-3-yl)-2,5,7,8-tetramethy l-chroman-6-ol; 2-(2,5-Dimethyl-thiophen-3-yl)-2,7,8-trimethyl-chroman-6-ol; 8-Chloro-2-(2,5-dimethyl-thiophen-3-yl)-2,5,7-trimethyl-chroman-6-ol; 5-Chloro-2,7,8-trimethyl-2-thiophen-2-yl-chroman-6-ol; 5-[3-(6-Mcthoxymcthoxy-2,7,8-trimcthyl-chroman-2-yl)-propylidcnc]-thiazolidinc-2,4-dionc; 5-[3-(6-Hydroxy-2,7,8-trimethyl-chroman-2-yl)-propylidene]-thiazolidine-2,4-dione; 3-[6-Hydroxy-2,7,8-trimethyl-2-(4,8,12-trimethyl- tridecyl)-chroman-5-yl-methylsulfanyl]-2-methyl-propionic acid; 2,7,8-Trimethyl-5-(5-methyl-1H-benzoimidazol-2-yl-sulfanylmethyl)-2-(4,8,12-trimethyl-tridecyl)-chroman-6-ol; 2-[6-Hydroxy-2,7,8-trimethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-5-ylmethylsulfanyl]-ethanesulfonic acid; 5-(4,6-Dimethyl-pyrimidin-2-ylsulfanylmethyl)-2,7 ,8-trimcthyl-2-(4,8,12-trimcthyl-tridccyl)-chroman-6-ol; 4-[2-(4,8-Dimcthyl-tridccyl)-6-hydroxy-2,7,8-trimethyl-chroman-5-ylmethylsulfanyl]-benzoic acid; 1-{3-[6-Hydroxy-2,7,8-trimethyl-2-(4,8,12-trimethyl-tridecyl)-chroman-5-ylmethylsulfanyl]-2-methyl-propionyl} -pyrrolidine-2-carboxylic acid; 2-(2,2-Dichloro-vinyl)-2,5,7,8-tetramethyl-chroman-6-ol; 2-(2,2-Dibromo-vinyl)-2,5,7,8-tetramethyl-chroman-6-ol; 5-(5-Chloro-3-methyl-pent-2-enyl)-2,2,7,8-tetramethyl-chroman-6-ol; 5-Chloro-2-(2,5-dimethyl-thiophen-3-yl)-2,7,8-trimethyl-chroman-6-ol; 2-(3-Chloro-propyl)-5,7-dimcthyl-2-thiophcn-2-yl-chroman-6-ol; 5-Chloro-2-(2,5-dimethyl-thiazol-4-yl)-2,7,8-trimethyl-chroman-6-ol; 5-Chloro-2-(2,5-dimethyl-thiazol-4-yl)-2,7,8-trimethyl-2H-chromen-6-ol; and 5-Chloro-2-(2,5-dimethyl-thiazol-4-yl)-2,7,8-trimethyl-chroman-6-ol.

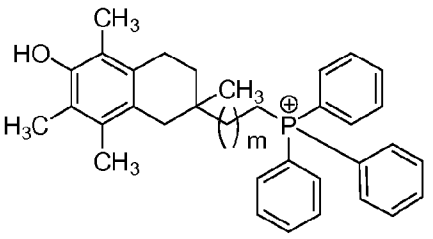
[0113] Compounds such as: dimebolin (2,8-dimethyl-5-(2-(6-methylpyridin-3-yl)ethyl)-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole), 8-chloro-2-methyl-5-(2-(6-methylpyridin-3-yl)ethyl)-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole, mebhydroline (5-benzyl-2-methyl-

2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole), 2,8-dimethyl-1,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3-b]indole, 8-fluoro-2-(3-(pyridin-3-yl)propyl)-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole, and 8-methyl-1,3,4,4a,5,9b-tetrahydro-1H-pyrido[4,3-b]indole.

[0114] Compounds such as: 2-(3-hydroxy-3-methylbutyl)-3,5-dimethyl-6-(4-(trifluoromethyl)phenyl)cyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-6-(4-methoxyphenyl)-3,5-dimethylcyclohexa-2,5-diene-1,4-dione; 4-(5-(3-hydroxy-3-methylbutyl)-2,4-dimethyl-3,6-dioxocyclohexa-1,4-dienyl)benzonitrile; 2-(3-hydroxy-3-methylbutyl)-3,5-dimethyl-6-(naphthalen-2-yl)cyclohexa-2,5-diene-1,4-dione; 2-(3,4-difluorophenyl)-6-(3-hydroxy-3-methylbutyl)-3,5-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(4-fluorophenyl)-6-(3-hydroxy-3-methylbutyl)-3,5-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(4-chlorophenyl)-6-(3-hydroxy-3-methylbutyl)-3,5-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(2,3-dihydrobenzofuran-2-yl)-6-(3-hydroxy-3-methylbutyl)-3,5-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-phenethylcyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-phenylcyclohexa-2,5-diene-1,4-dione; 2-benzyl-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-(3-phenylpropyl)cyclohexa-2,5-diene-1,4-dione; 2-(1-hydroxy-2-phenylethyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-3-(4-methoxyphenyl)-5,6-dimethyl-cyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-(4-(trifluoromethyl)-phenyl)cyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-(naphthalen-2-yl)cyclohexa-2,5-diene-1,4-dione; 2-(benzofuran-2-yl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(4-chlorophenyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(4-ethylphenyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-(3-(trifluoromethyl)phenyl)-cyclohexa-2,5-diene-1,4-dione; 2-(4-tert-butylphenyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-cyclohexa-2,5-diene-1,4-dione; 2-(4-fluorophenyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(3-fluorophenyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 4-(2-(3-hydroxy-3-methylbutyl)-4,5-dimethyl-3,6-dioxocyclohexa-1,4-dienyl)benzonitrile; 2-(3,4-difluorophenyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-cyclohexa-2,5-diene-1,4-dione; 2-(2-fluorophenyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-3-(3-methoxyphenyl)-5,6-dimethyl-cyclohexa-2,5-diene-1,4-dione; 2-(4-fluoro-2-methoxyphenyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(benzo[d][1,3]dioxol-5-yl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(2,4-difluorophenyl)-3-

(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-3-(4-methoxyphenyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(3,5-bis(trifluoromethyl)phenyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(4-chlorophenyl)-6-(3-hydroxy-3-methylbutyl)-3,5-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-(2-(thiazol-2-yl)ethyl)cyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-(2-(thiazol-5-yl)ethyl)cyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-(2-(pyridin-2-yl)ethyl)cyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-(2-(pyridazin-4-yl)ethyl)cyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-(2-(thiophen-2-yl)ethyl)cyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-(2-(thiophen-3-yl)ethyl)cyclohexa-2,5-diene-1,4-dione; 2-(2-(furan-2-yl)ethyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(2-(furan-3-yl)ethyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(2-(1H-pyrazol-5-yl)ethyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(2-(1H-pyrazol-4-yl)ethyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(2-(1H-pyrazol-1-yl)ethyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(2-(1H-imidazol-5-yl)ethyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(2-(1H-imidazol-2-yl)ethyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-(2-(oxazol-5-yl)ethyl)cyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-(2-(oxazol-2-yl)ethyl)cyclohexa-2,5-diene-1,4-dione; 2-(3-hydroxy-3-methylbutyl)-5,6-dimethyl-3-(2-(oxazol-4-yl)ethyl)cyclohexa-2,5-diene-1,4-dione; and 2-(2-(1H-indol-3-yl)ethyl)-3-(3-hydroxy-3-methylbutyl)-5,6-dimethylcyclohexa-2,5-diene-1,4-dione.

[0115] Compounds such as:



wherein m is $-C_1$-C_{20} alkyl, $-C_1$-C_{20} alkenyl, $-C_1$-C_{20} alkynyl, or $-C_1$-C_{20} containing at least one double bond and at least one triple bond, and the counterion is a pharmaceutically acceptable anion.

[0116] Compounds such as: 3-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)propyl triphenylphosphonium salts; 4-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)butyl triphenylphosphonium salts; 5-(4,5-dimethoxy-2-methyl-3,6-

dioxo-1,4-cyclohexadien-1-yl)pentyl triphenylphosphonium salts; 6-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)hexyl triphenylphosphonium salts; 7-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)heptyl triphenylphosphonium salts; 8-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)octyl triphenylphosphonium salts; 9-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)nonyl triphenylphosphonium salts; 10-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)decyl triphenylphosphonium salts; 11-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)undecyl triphenylphosphonium salts; 12-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)dodecyl triphenylphosphonium salts; 13-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)propyldecyl triphenylphosphonium salts; 14-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)butyldecyl triphenylphosphonium salts; 15-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)pentadecyl triphenylphosphonium salts; 16-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)hexadecyl triphenylphosphonium salts; 17-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)heptadecyl triphenylphosphonium salts; 18-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)octadecyl triphenylphosphonium salts; 19-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)nonadecyl triphenylphosphonium salts; 20-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)icosyl triphenylphosphonium salts; 3-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)propyl triphenylphosphonium salts; 4-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)butyl triphenylphosphonium salts; 5-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)pentyl triphenylphosphonium salts; 6-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)hexyl triphenylphosphonium salts; 7-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)heptyl triphenylphosphonium salts; 8-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)octyl triphenylphosphonium salts; 9-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)nonyl triphenylphosphonium salts; 10-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)decyl triphenylphosphonium salts; 11-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)undecyl triphenylphosphonium salts; 12-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)dodecyl triphenylphosphonium salts; 13-(4,5-dimethoxy-2-methyl-3,6-dihydroxybenzyl)propyldecyl triphenylphosphonium salts; 14-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)butyldecyl triphenylphosphonium salts; 15-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)pentadecyl triphenylphosphonium salts; 16-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)hexadecyl triphenylphosphonium salts; 17-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)heptadecyl triphenylphosphonium salts; 18-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)octadecyl triphenylphosphonium salts; 19-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)nonadecyl

triphenylphosphonium salts; 20-(4,5-dimethoxy-2-methyl-3,6-dihydroxyphenyl)icosyl triphenylphosphonium salts; wherein the counterion of the salt is a pharmaceutically acceptable anion such as bromide, methanesulfonate ethanesulfonate, propanesulfonate, benzenesulfonate, p-toluenesulfonate, or 2-naphthylene sulfonate.

[0117] Additionally, it is contemplated that coadministration of antioxidants could take the form of consuming foods known to have increased levels of beneficial antioxidants. Such foods include both regular foods and "superfoods" which contain antioxidants. These foods include fruits, vegetables, and other foodstuffs such as strawberries, blackcurrants, blackberries, oranges, blueberries, pomegranates, tea, coffee, olive oil, chocolate, cinnamon, herbs, red wine, grain cereals, eggs, meat, legumes, nuts, spinach, turnip, rhubarb, cocao beans, maize, beans, cabbage, and the like.

Delivery and Additional Formulations:

[0118] It is well known that triglycerides are the main constituents of vegetable oils and animal fats. It is also known that a triglyceride is an ester compound derived from glycerol and three fatty acids. Triglycerides are metabolized by enzymes such as lipases which hydrolyze ester bonds and release fatty acids and glycerol. Indeed, this metabolism releases fatty acids which can then be taken upon by cells via a fatty acid transporter protein. It is contemplated that PUFAs and PUFA mimetics that are useful in treating various diseases may be incorporated into fats such as triglycerides, diglycerides, and/or monoglycerides for administration to a patient.

[0119] The delivery of the PUFAs, PUFA mimetics, PUFA pro-drugs, and triglycerides containing PUFAs and/or PUFA mimetics could be through a modified diet. Alternatively, the PUFAs, PUFA mimetics, PUFA pro-drugs, and triglycerides containing PUFAs and/or PUFA mimetics can be administered as foods or food supplements, on their own or as complexes with 'carriers', including, but not limited to, complexes with albumin.

[0120] Other methods of delivering the reinforced PUFAs or their precursors, such as methods typically used for drug delivery and medication delivery, can also be employed. These methods include, but are not limited to, peroral delivery, topical delivery, transmucosal delivery such as nasal delivery, nasal delivery through cribriform plate, intravenous delivery, subcutaneous delivery, inhalation, or through eye drops.

[0121] Targeted delivery methods and sustained release methods, including, but not limited to, the liposome delivery method, can also be employed.

[0122] It is contemplated that the isotopically modified compounds described herein may be administered over a course of time, in which the cells and tissues of the subject will

contain increasing levels of isotopically modified compounds over the course of time in which the compounds are administered.

[0123] Compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, oil-in-water emulsions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Such compositions may contain excipients such as bulking agents, solubilization agents, taste masking agents, stabilizers, coloring agents, preservatives and other agents known to those ordinarily skilled in the art of pharmaceutical formulation. In addition, oral forms may include food or food supplements containing the compounds described herein. In some embodiments supplements can be tailor-made so that one type of PUFA, such as omega-3 or omega-6 fatty acids can be added to food or used as a supplement depending on the dominant fat that the food or the subject's diet contains. Moreover, compositions can be tailor-made depending on the disease to be treated. For example, an LDL related condition may require more D-linoleic acid because cardiolipin, which is made of linoleic acid, is oxidized. In other embodiments, such as retinal disease and neurological/CNS conditions may require more omega-3 fatty acids such as D-linolenic acid, because D-omega-3 fatty acids are more relevant for treating these diseases. In some aspects, when the disease is associated with HNE, then D-omega-6 fatty acids should be prescribed, whereas for HHE, D-omega-3 fatty acids should be prescribed.

[0124] Compositions may also be suitable for delivery by topical application, as a spray, cream, ointment, lotion, or as a component or additive to a patch, bandage or wound dressing. In addition the compound can be delivered to the site of the disease by mechanical means, or targeted to the site of the disease through the use of systemic targeting technologies such as liposomes (with or without chemical modification that provides them with affinity for the diseased tissue), antibodies, aptamers, lectins, or chemical ligands such as albumin, with affinity for aspects of the diseased tissue that are less abundant or not present on normal tissue. In some aspects, topical application of cosmetics may include the use of a carrier which is an isotopically modified compound or mimetic described herein for delivering through skin such as by a patch. Eye disorders may be treated with eyedrops.

[0125] A pharmaceutical composition may also be in a form suitable for administration by injection. Such compositions may be in the form of a solution, a suspension or an emulsion. Such compositions may include stabilizing agents, antimicrobial agents or other materials to improve the function of the medicament. Some aspects of the invention also encompass dry or desiccated forms of the compounds which can readily be formed or reconstituted into a solution suspension or emulsion suitable for administration by injection, or

for oral or topical use. Delivery by injection may be suitable for systemic delivery, and also local delivery such as injection into the eye for treating disorders relating to the eye.

Dosages

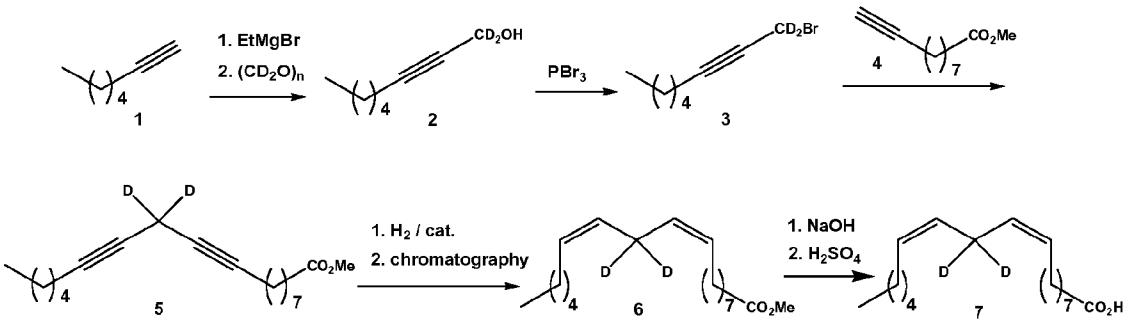
[0126] In some embodiments, compounds are dosed at about 0.01 mg/kg to about 1000 mg/kg, about 0.1 mg/kg to about 100 mg/kg, and/or about 1 mg/kg to about 10 mg/kg. In other embodiments, compounds are dosed at about: 0.01, 0.1, 1.0, 5.0, 10, 25, 50, 75, 100, 150, 200, 300, 400, 500, and/or 1000 mg/kg.

EXAMPLES

[0127] Experimental: MALDI-TOF mass-spectra were recorded on a PE-ABI Voyager Elite delayed extraction instrument. Spectra were acquired with an accelerating voltage of 25 KV and 100 ms delay in the positive ion mode. Unless otherwise specified, the 1H NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer. HPLC was carried out on a Waters system. Chemicals were from Sigma-Aldrich Chemical Company (USA), Avocado research chemicals (UK), Lancaster Synthesis Ltd (UK), and Acros Organics (Fisher Scientific, UK). Silica gel, TLC plates and solvents were from BDH/Merck. IR spectra were recorded with Vertex 70 spectrometer. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AC 400 instrument at 400 and 100 MHz respectively, in $CDCl_3$ (TMS at $\delta = 0.00$ or $CHCl_3$ at $\delta = 7.26$ for ^{1}H and $CHCl_3$ at $\delta = 77.0$ for ^{13}C as an internal standard).

[0128] One of ordinary skill in the art will recognize that the below described syntheses can be readily modified to prepare additional oxidation-resistant compounds. For example, one will recognize the ester of one type of stabilized compound can be cleaved to afford the corresponding carboxylic acid. Likewise, carboxylic acids can be readily converted into additional derivatives, such as esters. Additionally, one will appreciate that by varying the identity of the isotopically labeled starting materials, isotopic variants of the below described compounds may be prepared. In the below described syntheses, paraformaldehyde-d_2 is used as an isotopically labeled starting material. One will readily appreciate that the same synthetic transformations can be used with paraformaldehyde-d_1, formaldehyde-d_1, paraformaldehyde-d_2, formaldehyde-d_2, and carbon-13 labeled variants of the aforementioned compounds. Formaldehyde-d_1 is a well-characterized compound and is readily available from known sources such as formic acid-d_1, formic acid-d_2, and/or dichloromethane-d_1 using generally known and understood synthetic transformations. Furthermore, radioactive analogues of the compounds described herein can be prepared using tritium-containing starting materials. These compounds would be useful for determining incorporation in the cells and tissues of animals.

Example 1. Synthesis of 11,11-D2-linoleic acid



[0129] ***1,1-Dideutero-oct-2-yn-1-ol*** **(2)** To a solution of ethylmagnesium bromide prepared from bromoethane (100 ml), 1,2-dibromoethane (1 ml) and magnesium turnings (31.2 g) in dry THF (800 ml), heptyn-1 ((**1**); 170 ml) was added dropwise over 30-60 min under argon. The reaction mixture was stirred for 1 h, and then deuteroparaform (30 g) was carefully added in one portion. The reaction mixture was gently refluxed for 2 h, chilled to -10°C, and then 5-7 ml of water was slowly added. The mixture was poured into 0.5 kg slurry of crushed ice and 40 ml concentrated sulphuric acid and washed with 0.5 L of hexane. The organic phase was separated, and the remaining aqueous phase was extracted with 5:1 hexane:ethyl acetate (3 x 300 ml). The combined organic fraction was washed with sat. NaCl (1 x 50 ml), sat. $NaHCO_3$, (1 x 50 ml), and dried over Na_2SO_4. The solvent was evaporated *in vacuo* to yield 119.3 g (99%) of colourless oil which was used without further purification. HRMS, *m/z* calculated for $C_8H_{12}D_2O$: 128.1168; found: 128.1173. ^1H NMR ($CDCl_3$, δ): 2.18 (t, J = 7.0, 2H), 1.57 (s, 1H), 1.47 (q, J = 7.0 Hz, 2H), 1.31 (m, 4H), 0.87 (t, J = 7.0 Hz, 3H).

[0130] ***1,1-Dideutero-1-bromo-oct-2-yne*** **(3)** To a solution of (**2**) (3.48 g; 27.2 mmol) and pyridine (19 ml) in dry diethyl ether (300 ml), 36 ml of PBr_3 in 35 ml diethyl ether was added dropwise with stirring over 30 min at -15°C under argon. The reaction mixture was allowed to gradually warm up to r.t. and then refluxed 3 h with stirring and 1 h without stirring. The reaction mixture was then cooled down to -10°C and 500 ml of cold water was added. When the residue dissolved, saturated NaCl (250 ml) and hexane (250 ml) were added, and the organic layer was separated. The aqueous fraction was washed with hexane (2 x 100 ml), and the combined organic fractions were washed with NaCl (2 x 100 ml) and dried over Na_2SO_4 in presence of traces of hydroquinone and triethylamine. The solvent was removed by distillation at atmospheric pressure followed by rotary evaporation. The residue was fractionated by vacuum distillation (3 mm Hg) to give 147.4 g (82 % counting per deutero-paraform) of pale yellow oil. B.p. 75°C. HRMS, *m/z* calculated for $C_8H_{11}D_2Br$: 190.0324; found: 189.0301,

191.0321. ^{1}H NMR ($CDCl_3$, δ): 2.23 (t, J = 7.0 Hz, 2H, CH_2), 1.50 (m, 2H, CH_2), 1.33 (m, 4H, CH_2), 0.89 (t, J = 6.9 Hz, 3H, CH_3),

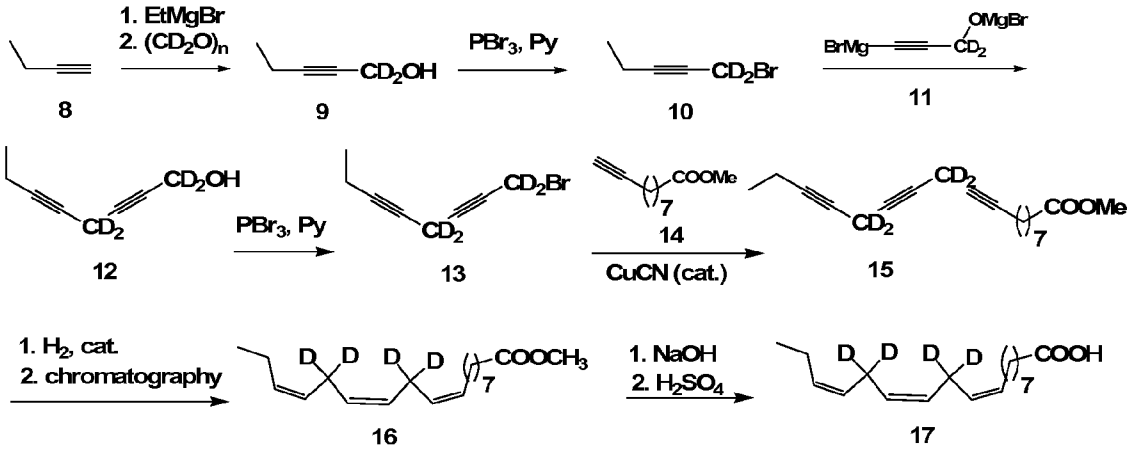
[0131] ***11,11-Dideutero-octadeca-9,12-diynoic acid methyl ester*** **(5)** CuI (133 g) was quickly added to 400 ml of DMF (freshly distilled over CaH_2), followed by dry NaI (106 g), K_2CO_3 (143 g). Dec-9-ynoic acid methyl ester ((**4**); 65 g) was then added in one portion, followed by bromide (**3**) (67 g). Additional 250 ml of DMF was used to rinse the reagents off the flask walls into the bulk of reaction mixture, which was then stirred for 12 h. 500 ml of saturated aqueous NH_4Cl was then added with stirring, followed in a few minutes by saturated aqueous NaCl and then by a 5:1 mixture of hexane:EtOAc (300 ml). The mixture was further stirred for 15 min and then filtered through a fine mesh Schott glass filter. The residue was washed with hexane:EtOAc mix several times. The organic fraction was separated, and the aqueous phase was additionally extracted (3 x 200 ml). The combined organic fraction was dried (Na_2SO_4), traces of hydroquinone and diphenylamine were added, and the solvent was evaporated *in vacuo*. The residue was immediately distilled at 1 mm Hg, to give 79 g (77%) of a 165-175°C boiling fraction. HRMS, *m/z* calculated for $C_{19}H_{28}D_2O_2$: 292.2369; found: 292.2365. ^{1}H NMR ($CDCl_3$, δ): 3.67(s,3H,OCH_3),2.3 (t,J = 7.5 Hz, 2H, CH_2),2.14 (t, J = 7.0 Hz, 4H, CH_2), 1.63 (m, 2H, CH_2), 1.47 (m, 4H, CH_2), 1.3 (m, 10H, CH_2), 0.88 (t, J = 7.0 Hz, 3H, CH_3).

[0132] ***11,11-Dideutero-cis,cis-octadeca-9,12-dienoic acid methyl ester*** **(6)** A suspension of nickel acetate tetrahydrate (31.5 g) in 96 % EtOH (400 ml) was heated with stirring to approx. 50-60°C until the salt dissolved. The flask was flushed with hydrogen, and then 130 ml of $NaBH_4$ solution, (prepared by a 15 min stirring of $NaBH_4$ suspension (7.2 g) in EtOH (170 ml) followed by filtering) was added dropwise over 20-30 min with stirring. In 15-20 min ethylenediamine (39 ml) was added in one portion, followed in 5 min by an addition of (**5**) (75 g) in EtOH (200 ml). The reaction mixture was very vigorously stirred under hydrogen (1 atm). The absorption of hydrogen stopped in about 2 h. To the reaction mixture, 900 ml of hexane and 55 ml of ice cold AcOH were added, followed by water (15 ml). Hexane (400 ml) was added, and the mixture was allowed to separate. Aqueous fractions were extracted by 5:1 mix of hexane:EtOAc. The completion of extraction was monitored by TLC. The combined organic phase was washed with diluted solution of H_2SO_4, followed by saturated $NaHCO_3$ and saturated NaCl, and then dried over Na_2SO_4. The solvent was removed at reduced pressure. Silica gel (Silica gel 60, Merck; 162 g) was added to a solution of silver nitrate (43 g) in anhydrous MeCN (360 ml), and the solvent removed on a rotavap. The obtained impregnated silica gel was dried for 3 h at 50°C (aspiration pump) and then 8 h on an oil pump. 30 g of this silica was used per gram of product. The reaction mixture was dissolved in a small volume of

hexane and applied to the silver-modified silica gel, and pre-washed with a 1-3 % gradient of EtOAc. When the non-polar contaminants were washed off (control by TLC), the product was eluted with 10 % EtOAc and the solvent evaporated in vacuo to give 52 g of the title ester (**6**) as a colourless liquid. HRMS, *m/z* calculated for $C_{19}H_{32}D_2O_2$: 296.2682; found: 296.2676. IR (CCl_4): $\tilde{\nu}$ = 1740 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 5.32 (m, 4H), 3.66 (s, 3H, OCH_3), 2.29 (t, J = 7.5 Hz, 2H, CH_2), 2.02 (m, 4H, CH_2), 1.60 (m, 2H, CH_2), 1.30 (m, 14H, CH_2), 0.88 (t, J = 7.0 Hz, 3H, CH_3).

[0133] ***11,11-Dideutero-cis,cis-octadeca-9,12-dienoic acid*** (**7**) A solution of KOH (46 g) in water (115 ml) was added to a solution of ester (**6**) (46 g) in MeOH (60 ml). The reaction mixture was stirred at 40-50°C for 2 h (control by TLC) and then diluted with 200 ml of water. Two thirds of the solvent were removed (rotavap). Diluted sulphuric acid was added to the residue to pH 2, followed by diethyl ether with a little pentane. The organic layer was separated and the aqueous layer washed with diethyl ether with a little pentane. The combined organic fractions were washed with saturated aqueous NaCl and then dried over Na_2SO_4. The solvent was evaporated to give 43 g of (**7**) (99%). IR (CCl_4): $\tilde{\nu}$ = 1741, 1711 cm^{-1}.

Example 2. Synthesis of 11,11,14,14-D4-linolenic acid



[0134] ***1,1-Dideutero-pent-2-yn-1-ol*** (**9**) But-1-yne (**8**) was slowly bubbled through a solution of ethylmagnesium bromide prepared from bromoethane (100 ml) and magnesium turnings (31.3 g) in dry THF (800 ml) on a bath (-5°C). Every now and then the bubbling was stopped and the cylinder with but-1-yne was weighed to measure the rate of consumption. The supply of alkyne was stopped shortly after a voluminous precipitate formed (the measured mass of alkyne consumed was 125 g). The reaction mixture was warmed up to r.t. over 30 min, and then stirred for 15 min. The mixture was then heated up to 30°C, at which point the precipitate dissolved, and then stirred at r.t. for another 30 min. Deuteroparaform (28 g) was added in one portion and the mixture was refluxed for 3 h, forming a clear solution. It was cooled down to r.t.

and poured into a mixture of crushed ice (800 g) and 50 ml conc. H_2SO_4. Hexane (400 ml) was added and the organic layer was separated. The aqueous phase was saturated with NaCl and extracted with a 4:1 mixture of hexane:EtOAc (1 L). The completion of extraction process was monitored by TLC. The combined organic phases were washed with saturated NaCl, $NaHCO_3$ and again NaCl, and dried over Na_2SO_4. The solvent was removed by distillation at the atmospheric pressure (max vapour temperature 105°C). The residue (70.5 g; 94 %) was used without further purification. HRMS, *m/z* calculated for $C_5H_6D_2O$: 86.0699; found: 86.0751. ^1H NMR ($CDCl_3$, δ): 2.21 (q, J = 7.5 Hz, 2H, CH_2), 1.93 (br s, 1H, OH), 1.12 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ): 87.7, 77.6, 13.7, 12.3 (signal of CD_2 is absent).

[0135] ***1,1-Dideutero-1-bromo-pent-2-yne*** **(10)** To a solution of **(9)** (70.5 g) and pyridine (16.5 ml) in dry diethyl ether (280 ml), 32.3 ml of PBr_3 in 50 ml diethyl ether was added dropwise with stirring over 30 min at -10°C under argon. The reaction mixture was allowed to gradually warm up to r.t. over 1 h. A small amount of hydroquinone was added, and the mixture was then refluxed for 4.5 h. The reaction mixture was then cooled down to -10°C and 350 ml of cold water was added. When the residue dissolved, saturated NaCl (350 ml) and hexane (300 ml) were added, and the organic layer was separated. The aqueous fraction was washed with diethyl ether (2 x 150 ml), and the combined organic fractions were washed with NaCl (2 x 50 ml) and dried over Na_2SO_4 in presence of traces of hydroquinone and triethylamine. The solvent was removed at atmospheric pressure, and then the 147-155°C boiling fraction was distilled off. Alternatively, upon reaching 100°C, the distillation at atmospheric pressure was stopped and the product distilled off at 77-84°C (25 mm Hg). Yield: 107 g of clear liquid. HRMS, *m/z* calculated for $C_5H_5D_2Br$: 147.9855; found: 146.9814, 148.9835. IR (CCl_4): $\tilde{\nu}$ = 2251 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 2.23 (q, J = 7.5 Hz, 2H, CH_2), 1.11 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ): 89.3, 74.5, 13.4, 12.6 (signal of CD_2 is absent).

[0136] ***1,1,4,4-Tetradeutero-octa-2,5-diyn-1-ol*** **(12)** Ethylmagnesium bromide, prepared from ethyl bromide (53 ml) and magnesium turnings (15.8 g) in 400 ml of dry THF, was added in small portions to 350 ml of dry THF, simultaneously with acetylene bubbling through this mixture (at approx. 25 L/h rate) with vigorous stirring. The Grignard reagent solution was fed to the mixture at approx. 10 ml per 2-5 min. When all ethylmagnesium bromide was added (after approx. 2.5 h), acetylene was bubbled through the system for another 15 min. Deuteroparaform (17.3 g) and CuCl (0.2 g) were added under argon, and the reaction mixture was refluxed without stirring for 2.5 h, until deuteroparaform dissolved, to yield a solution of **(11)**. Ethylmagnesium bromide solution, prepared from 14.8 g magnesium and 50 ml ethyl bromide in 250 ml of dry THF, was added dropwise to the reaction mixture over 20

min. When the gas emanation ceased, a condenser was attached and 250 ml of solvent were distilled off. The reaction mixture was then cooled to 30°C, and CuCl (1.4 g) was added followed by a dropwise addition, over 15 min, of bromide (**10**) (69 g). The reaction mixture was then refluxed for 5 h, cooled slightly (a precipitate will form if cooling is too fast), and poured into a slurry of crushed ice (1-1.2 kg) and 40 ml concentrated H_2SO_4. The mixture was washed with hexane (600 ml). The organic fraction was separated, and the aqueous fraction was additionally extracted with 5:1 hexane:EtOAc (2 x 400 ml). The combined organic fraction was washed, with saturated NaCl, followed by saturated $NaHCO_3$ and NaCl. The bulk of the solvent was removed at atmospheric pressure in presence of traces of hydroquinone and triethylamine. The residue was flushed through 100 ml of silica gel (eluent: 7:1 hexane:EtOAc). The bulk of the solvent was removed at the atmospheric pressure, and the remainder on a rotavap. 49.5 g (85 %) of the title compound obtained was used without further purification. HRMS, *m/z* calculated for $C_8H_6D_4O$: 126.0979; found: 126.0899. IR(CCl_4): $\tilde{\nu}$=3622 cm^{-1}. ^1H NMR($CDCl_3$,δ):2.16(q,J=7.5Hz,2H, CH_2), 1.85 (br s, 1 H, OH), 1.11 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ): 82.3, 80.4, 78.3, 72.6, 13.7, 12.2

[0137] ***1,1,4,4-Tetradeutero-1-bromo-octa-2,5-diyne*** (**13**) was synthesized as described for bromide (**3**); 2 ml of pyridine, 14 ml PBr_3 and 250 ml of diethyl ether was used for 54.2 g of alcohol (**12**). The product was purified by distillation at 4 mm Hg. Yield: 53 g (65 %) of (**13**); b.p. 100-110°C. HRMS, *m/z* calculated for $C_8H_5D_4Br$: 188.0135; found: 187.0136, 189.0143. IR (CCl_4): $\tilde{\nu}$ = 2255 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 2.13 (q, J = 7.5 Hz, 2H, CH_2); 1.07 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ): 82.5, 81.8, 75.0, 72.0, 13.6, 12.2.

[0138] ***11,11,14,14-Tetradeutero-octadeca-8,12,15-triynoic acid methyl ester*** (**15**) was synthesized in a way similar to that described for 11,11-dideutero-octadeca-9,12-diynoic acid methyl ester (**5**). CuI (97 g) was quickly added to 400 ml of DMF (freshly distilled over CaH_2), followed by dry NaI (77.5 g), K_2CO_3 (104.5 g). Dec-9-ynoic acid methyl ester ((**14**); 47.5 g) was then added in one portion, followed by bromide (**13**) (48.5 g). Additional 250 ml of DMF was used to rinse the reagents off the flask walls into the bulk of reaction mixture, which was then stirred for 12 h. 500 ml of saturated aqueous NH_4Cl was then added with stirring, followed in a few minutes by saturated aqueous NaCl (300 ml) followed by a 5:1 mixture of hexane:EtOAc (300 ml). The mixture was further stirred for 15 min and then filtered through a fine mesh Schott glass filter. The residue was washed with hexane:EtOAc mix several times. The organic fraction was separated, and the aqueous phase was additionally extracted (3 x 200 ml). The combined organic fraction was dried (Na_2SO_4), traces of hydroquinone and diphenylamine were added, and the solvent was evaporated *in vacuo*. The residue was

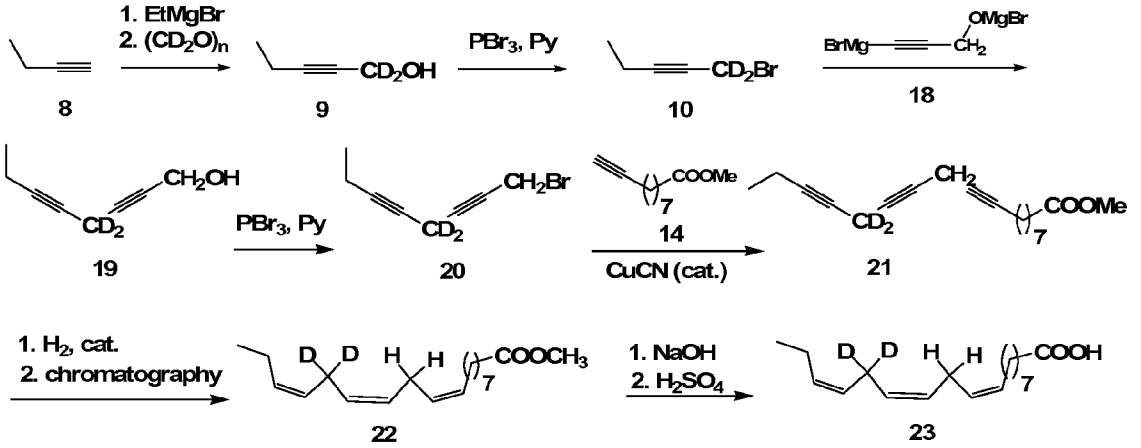
immediately distilled at 1 mm Hg, to give 45.8 g (62%) of a 173-180°C boiling fraction. An additional crystallisation was carried out as follows. The ester (**15**) was dissolved in hexane (500 ml) and cooled down to -50°C. The crystals formed were washed in cold hexane. The yield of this step is 80 %. HRMS, *m/z* calculated for $C_{19}H_{22}D_4O_2$: 290.2180; found: 290.2200. ^1H NMR ($CDCl_3$, δ): 3.66 (s, 3H, OCH_3), 2.29 (t, J = 7.5 Hz, 2H, CH_2), 2.15 (m, 4H, CH_2), 1.61 (m, 2H, CH_2), 1.47 (m, 2H, CH_2), 1.30 (m, 6H, CH_2), 1.11 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ): 174.1, 82.0, 80.6, 74.7, 74.6, 73.7, 73.0, 51.3, 33.9, 28.9, 28.6, 28.52, 28.49, 24.8, 18.5, 13.7, 12.2.

[0139] ***11,11,14,14-Tetradeutero-cis,cis,cis-octadeca-8,12,15-trienoic acid methyl ester*** (**16**) was synthesized in a way similar to that described for 11,11-Dideutero-cis,cis-octadeca-9,12-dienoic acid methyl ester ('**6**'). A suspension of nickel acetate tetrahydrate (42 g) in 96 % EtOH (400 ml) was heated with stirring to approx. 50-60°C until the salt dissolved. The flask was flushed with hydrogen, and then 130 ml of $NaBH_4$ solution, (prepared by a 15 min stirring of $NaBH_4$ suspension (7.2 g) in EtOH (170 ml) followed by filtering) was added dropwise over 20-30 min with stirring. In 15-20 min ethylenediamine (52 ml) was added in one portion, followed in 5 min by an addition of (**15**) (73 g) in EtOH (200 ml). The reaction mixture was very vigorously stirred under hydrogen (1 atm). The absorption of hydrogen stopped in about 2 h. To the reaction mixture, 900 ml of hexane and 55 ml of ice cold AcOH were added, followed by water (15 ml). Hexane (400 ml) was added, and the mixture was allowed to separate. Aqueous fractions were extracted by 5:1 mix of hexane:EtOAc. The completion of extraction was monitored by TLC. The combined organic phase was washed with diluted solution of H_2SO_4, followed by saturated $NaHCO_3$ and saturated NaCl, and then dried over Na_2SO_4. The solvent was removed at reduced pressure. Silica gel for purification was prepared as described for (**6**). 30 g of this silica was used per gram of product. The reaction mixture was dissolved in a small volume of hexane and applied to the silver-modified silica gel, and pre-washed with a 1-5 % gradient of EtOAc. When the non-polar contaminants were washed off (control by TLC), the product was eluted with 10 % EtOAc and the solvent evaporated in vacuo to give 42 g of the title ester (**16**) as a colourless liquid. HRMS, *m/z* calculated for $C_{19}H_{28}D_4O_2$: 296.2649; found: 296.2652. IR (CCl_4): $\tilde{\nu}$ = 1740 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 5.4 (m, 6H, CH-double bond), 3.68 (s, 3H, OCH_3), 2.33 (t, J = 7.5 Hz, 2H, CH_2), 2.09 (m, 4H, CH_2), 1.62 (m, 2H, CH_2), 1.33 (m, 8H, CH_2), 0.97 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ): 174.1, 131.9, 130.2, 128.2, 128.1, 127.7, 126.9, 51.3, 34.0, 29.5, 29.04, 29.02, 27.1, 25.5, 24.9, 20.5, 14.2.

[0140] ***11,11,14,14-Tetradeutero-cis,cis,cis-octadeca-8,12,15-trienoic acid*** (**17**) A solution of KOH (1.5 g, 27 mmol) in water (2.6 ml was added to a solution of ester (**16**) (1.00 g,

3.4 mmol) in MeOH (15 ml). The reaction mixture was stirred at 40-50°C for 2 h (control by TLC) and then diluted with 20 ml of water. Two thirds of the solvent were removed (rotavap). Diluted sulfuric acid was added to the residue to pH 2, followed by diethyl ether with a little pentane (50 ml). The organic layer was separated and the aqueous layer washed with diethyl ether with a little pentane (3 x 30 ml). The combined organic fractions were washed with saturated aqueous NaCl and then dried over Na_2SO_4. The solvent was evaporated to give 0.95 g of (**17**) (100%). IR (CCl_4): $\tilde{\nu}$ = 1741, 1711 cm^{-1}.

Example 3. Synthesis of 14,14-D2-linolenic acid



[0141] ***4,4-Dideutero-octa-2,5-diyn-1-ol*** **(19)** To a solution of ethylmagnesium bromide, prepared from ethyl bromide (9.2 ml, 123.4 mmol) and magnesium turnings (2.74 g, 112.8 mmol) in 40 ml of dry THF, on an ice bath with stirring, propargyl alcohol (3.16 g, 56.4 mmol) in THF (5 ml) was added dropwise over 10-15 min. The reaction mixture was allowed to warm up to r.t. and stirred for another 2 h, with occasional warming to 40°C. To thus generated dianion, 0.13g of CuCl was added, followed by slow (over 15 min) addition of bromide (10) (6.9 g) in THF (20 ml). The reaction mixture was then stirred for 1 h at r.t. and then refluxed for 5 h. The reaction mixture was then refluxed for 5 h, cooled slightly (a precipitate will form if cooling is too fast), and poured into a slurry of crushed ice and 2.5 ml concentrated H2SO4. The mixture was washed with hexane (600 ml). The organic fraction was separated, and the aqueous fraction was additionally extracted with 5:1 hexane:EtOAc. The combined organic fraction was washed, with saturated NaCl, followed by saturated NaHCO3 and NaCl, and dried over Na2SO4. The bulk of the solvent was removed at atmospheric pressure in presence of traces of hydroquinone and triethylamine. The product was purified by CC (hexane:EtOAc = 15:1) to give 3.45 g (59 %) of the product 19. HRMS, m/z calculated for C8H8D2O: 124.0855; found: 124.0849. IR (CCl4): $\tilde{\nu}$ = 3622 cm-1. 1H NMR (CDCl3, δ): 4.21 (m, 2H, CH2), 2.4 (m, 1H, OH), 2.16 (q, J = 7.5 Hz, 2H, CH2), 1.11 (t, J = 7.5 Hz, 3H, CH3). 13C NMR (CDCl3, δ): 82.3, 80.4, 78.3, 72.6, 51.0, 13.7, 12.2.

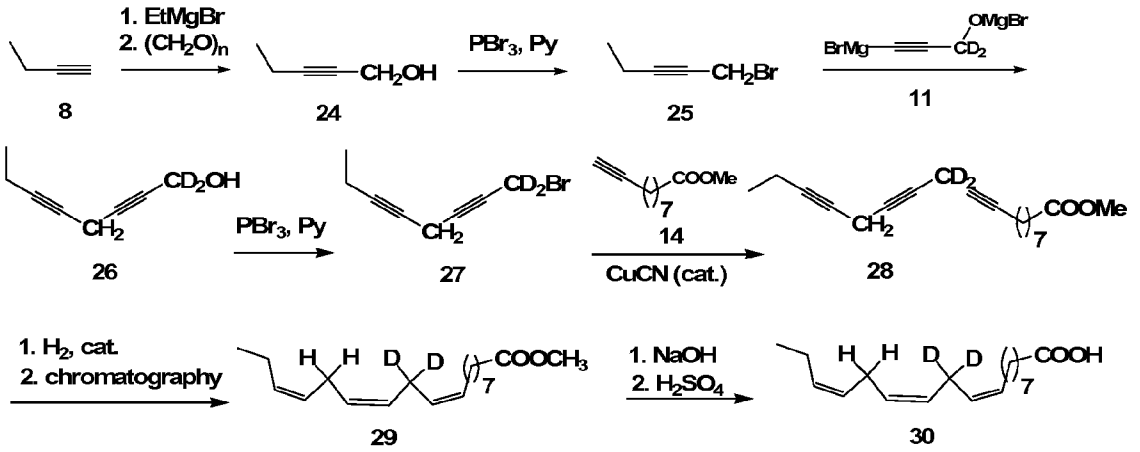
[0142] ***4,4-Dideutero-1-bromo-octa-2,5-diyne*** (**20**) was synthesized as described for (**3**), except all solvent was removed on a rotavap. From 3.4 g (27 mmol) of (**19**), 3.9 g (75 %) of the bromide (**20**) was obtained, which was used without further purification. HRMS, m/z calculated for $C_8H_7D_2Br$: 186.0011; found: 185.0019, 187.0012. IR (CCl_4): $\tilde{\nu}$ = 2255 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 3.88 (br s, 2H, CH_2), 2.13 (q, J = 7.5 Hz, 2H, CH_2), 1.07 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ): 82.5, 81.8, 75.0, 72.0, 14.8, 13.6, 12.2.

[0143] ***14,14-Dideutero-octadeca-8,12,15-triynoic acid methyl ester*** (**21**) was synthesized as described for (**5**). The product obtained from 9.7 g CuI, 7.8 g NaI, 10.5 g K_2CO_3, 4.85 g of bromide (**20**), 4.75 g of methyl ester (**14**) and 40 ml of anhydrous DMF, was purified by CC (25:1 hexane:EtOAc) to give 4.5 g (60%) of the title compound. HRMS, *m/z* calculated for $C_{19}H_{24}D_2O_2$: 288.2056; found: 288.2046. ^1H NMR ($CDCl_3$, δ): 3.66 (s, 3H, OCH_3), 3.12 (m, 2H, CH_2), 2.29 (t, J = 7.5 Hz, 2H, CH_2), 2.15 (m, 4H, CH_2), 1.61 (m, 2H, CH_2), 1.47 (m, 2H, CH_2), 1.30 (m, 6H, CH_2), 1.11 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ): 174.1, 82.0, 80.6, 74.7, 74.6, 73.7, 73.0, 51.3, 33.9, 28.9, 28.6, 28.52, 28.49, 24.8, 18.5, 13.7, 12.2, 9.7.

[0144] ***14,14-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid methyl ester*** (**22**) was synthesized as described for the linoleic acid derivative (**6**). For a reduction of 4.5 g of (**21**), 2.6 g of nickel acetate tetrahydrate and 3.2 ml ethylenediamine was used. The product was purified on $AgNO_3$-impregnated silica gel as described for (**6**). HRMS, *m/z* calculated for $C_{19}H_{30}D_2O_2$: 294.2526; found: 294.2529. IR (CCl_4): $\tilde{\nu}$ = 1740 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 5.37 (m, 6H, CH-double bond), 3.68 (s, 3H, OCH_3), 2.82 (m, 2H, CH_2), 2.33 (t, J = 7.5 Hz, 2H, CH_2), 2.09 (m, 4H, CH_2), 1.62 (m, 2H, CH_2), 1.33 (m, 8H, CH_2), 0.97 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ): 174.1, 131.9, 130.2, 128.2, 128.1, 127.7, 126.9, 51.3, 34.0, 29.5, 29.1, 29.04, 29.02, 27.1, 25.5, 24.9, 20.5, 14.2.

[0145] ***14,14-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid*** (**23**) To a solution of (**22**) (1 g, 3.4 mmol) in MeOH (15 ml), a solution of KOH (1.5 g, 27 mmol) in water (2.6 ml) was added in one portion. The reaction mixture was then processed as described for (**7**) to yield 0.94g (99 %) of the title acid. IR (CCl_4): $\tilde{\nu}$ = 1741, 1711 cm^{-1}.

Example 4. Synthesis of 11,11-D2-linolenic acid



[0146] ***Pent-2-yn-1-ol*** **(24)** Butyn-1 ((**8**); 10.4 g) was bubbled through an ice-cold solution prepared from bromoethane (11.2 ml) and magnesium turnings (3.6 g) in THF (100 ml). The reaction mixture was allowed to warm up to r.t. and then stirred for 15 min. The mixture was then heated up to 30°C, at which point all precipitate dissolved. The heating was removed and the mixture stirred for another 30 min, and then paraform (3 g) was added in one portion. The reaction mixture was refluxed for 3 h (all paraform dissolved), then cooled to r.t., poured into a mixture of crushed ice (80 g) and 8 ml conc. H_2SO_4, and extracted with diethyl ether. The organic phase was washed with saturated $NaHCO_3$ and NaCl, and dried over Na_2SO_4. The solvent was removed on a rotavap, and the residue (7.56 g; 90 %) was used without further purification. HRMS, m/z calculated for C_5H_8O: 84.0575; found: 84.0583.

[0147] ***1-Bromo-pent-2-yne*** **(25)** To a solution of (**24**) (11.7 g) and pyridine (2.66 ml) in dry diethyl ether (34 ml), 5.2 ml of PBr_3 in 5 ml diethyl ether was added dropwise with stirring over 30 min at -10°C under argon. The reaction mixture was allowed to gradually warm up to r.t. over 1 h. A catalytic amount of hydroquinone was added, and the mixture was then refluxed for 4.5 h. The reaction mixture was then cooled down to -10°C and 35 ml of cold water was added. When the residue dissolved, saturated NaCl (35 ml) and diethyl ether (30 ml) were added, and the organic layer was separated. The aqueous fraction was washed with diethyl ether (2 x 15 ml), and the combined organic fractions were washed with NaCl (2 x 400 ml) and dried over $MgSO_4$. The solvent was removed at atmospheric pressure, and then under reduced pressure (25 mm Hg), the 60-90°C fraction was collected. Yield: 11.1 g (84 %). HRMS, *m/z* calculated for C_5H_7Br: 145.9731; found: 144.9750, 146.9757.

[0148] ***1,1-Dideutero-octa-2,5-diyn-1-ol*** **(26)** was synthesized as described for (**12**) with 87 % yield. HRMS, m/z calculated for $C_8H_8D_2O$: 124.0855;found:124.0868. IR (CCl_4):

$\tilde{\nu}$ = 3622 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 2.65 (m, 2H, CH_2), 2.4 (m, 1H, OH), 2.1 (q, 2H, CH_2), 1.09 (t, 3H, CH_3).

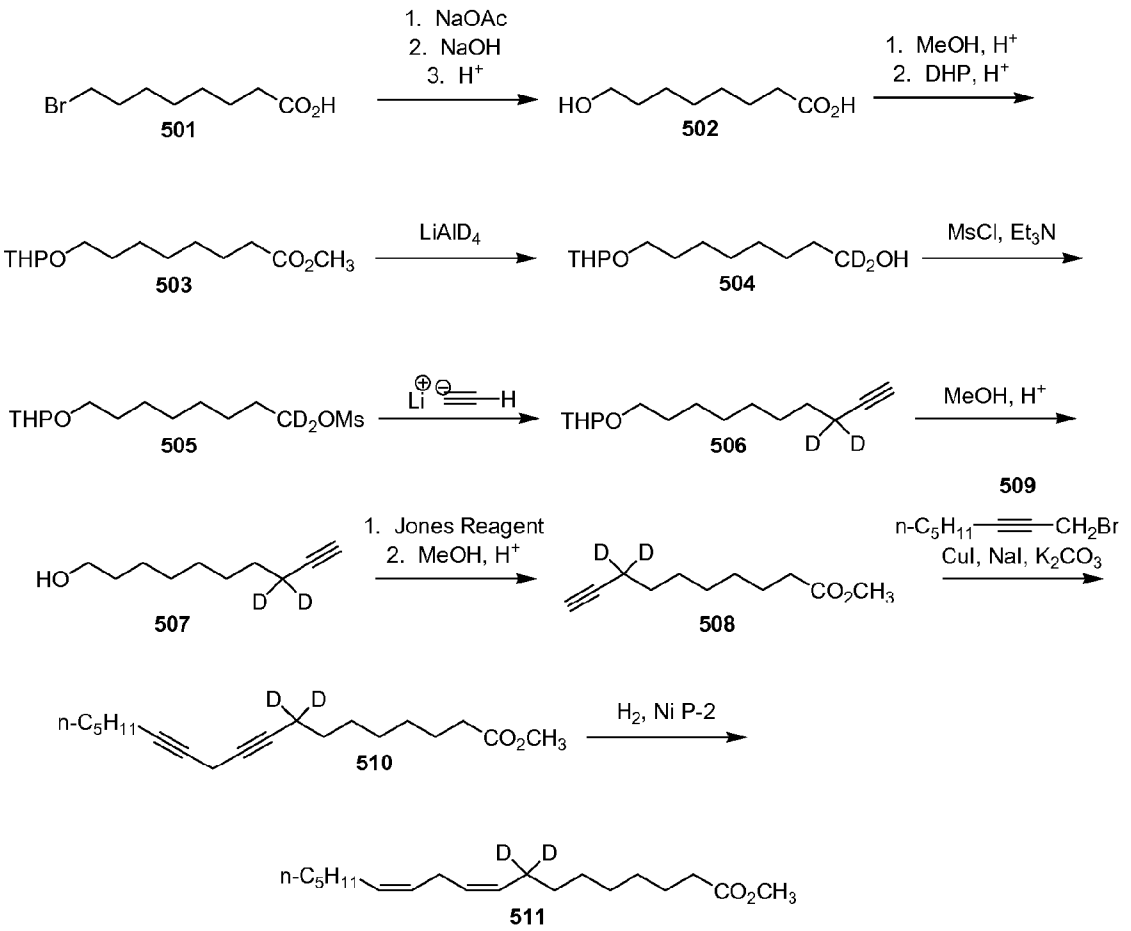
[0149] ***1,1-Dideutero-1-bromo-octa-2,5-diyne*** (**27**) was synthesized as described for (**3**), except all solvent was removed on a rotavap. The product was purified by distillation at reduced pressure. Yield: 86 % (b.p. 100-105°C at 4 mm Hg). HRMS, m/z calculated for $C_8H_7D_2Br$: 186.0011; found: 184.9948, 187.9999. IR (CCl_4): $\tilde{\nu}$ = 2255 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 2.66 (m, 2H, CH_2), 2.1 (q, 2H, CH_2), 1.09 (t, 3H, CH_3).

[0150] ***11,11-Dideutero-octadeca-8,12,15-triynoic acid methyl ester*** (**28**) was synthesized as described for (**5**). The product obtained from 7.1 g CuI, 5.66 g NaI, 7.65 g K_2CO_3, 3.55 g of bromide (**27**), 3.47 g of methyl ester (**14**) and 30 ml of anhydrous DMF, was purified by CC (25:1 hexane:EtOAc) to give 3.7 g of the title compound. HRMS, m/z calculated for $C_{19}H_{24}D_2O_2$: 288.2056; found: 288.2069. ^1H NMR ($CDCl_3$, δ): 3.7 (s, 3H, OCH_3), 3.15 (br. s, 2H, CH_2), 2.35 (m, 2H, CH_2), 2.17 (m, 4H, CH_2), 1.61 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 1.35 (m, 6H, CH_2), 1.11 (t, 3H, CH_3).

[0151] ***11,11-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid methyl ester*** (**29**) was synthesized as described for the linoleic acid derivative (**6**). For a reduction of 3.7 g of (**28**), 2.16 g of nickel acetate tetrahydrate and 2.62 ml ethylenediamine was used. The product was purified on $AgNO_3$-impregnated silica gel as described for (**6**) to give 1.5 g. HRMS, m/z calculated for $C_{19}H_{30}D_2O_2$: 294.2526; found: 294.2402. IR (CCl_4): $\tilde{\nu}$ = 1740 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 5.37 (m, 6H, CH-double bond), 3.6 (s, 3H, OCH_3), 2.82 (m, 2H, CH_2), 2.33 (t, o = 7.5 Hz, 2H, CH_2), 2.09 (m 4H, CH_2), 1.62 (m, 2H, CH_2), 1.33 (m, 8H, CH_2), 0.97 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ): 174.1, 131.9, 130.2, 128.2, 128.1, 127.7, 126.9, 51.3, 34.0, 29.5, 29.1, 29.04, 29.02, 27.1, 25.5, 24.9, 20.5, 14.2.

[0152] ***11,11-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid*** (**30**) To a solution of (29) (1.5 g, 5.1 mmol) in MeOH (7.5 ml), a solution of KOH (1.5 g, 27 mmol) in water (3 ml) was added in one portion. The reaction mixture was then processed as described for (17) to yield 0,9 g of the title acid. IR (CCl_4): $\tilde{\nu}$ = 1741, 1711 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 11.2 (br s, 1 H, COOH), 5.37 (m, 6H, CH-double bond), 2.83 (m, 2H, CH_2), 2.35 (t, J = 7.5 Hz, 2H, CH_2), 2.06 (m 4H, CH_2), 1.63 (m, 2H, CH_2), 1.32 (m, 8H, CH_2), 0.97 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, δ): 180.4, 131.9, 130.2, 128.3, 128.1, 127.6, 127.1, 34.1, 29.5, 29.1, 29.03, 28.98, 27.2, 25.5, 24.6, 20.5, 14.2.

Example 5. Synthesis of 8,8-D_2-Linoleic Acid Methyl Ester



[0153] 8-Hydroxyoctanoic acid (502). A solution of 8-bromocaprylic acid (**501**, 37.5 g, 168 mmol), anhydrous sodium acetate (60.0 g, 732 mmol) and sodium iodide (1.0 g, 6.7 mmol) in DMF (200 ml) was stirred at 110-120°C for 8 h. The reaction mixture was cooled to r.t., a solution of potassium hydroxide (28 g, 0.5 mol) in water (150 ml), was added, and the mixture was stirred at 100°C for another hour. The reaction mixture was cooled to r.t. and poured into slurry of ice and concentrated sulfuric acid (45 ml). The solution obtained was saturated with NaCl and extracted (9 x 150 ml) with a mixture of EtOAc and petroleum ether (1:1). Combined organic fractions were washed twice with saturated NaCl and dried over Na_2SO_4. The solvent was evaporated to give 26.5 g (98%) of the product which was used without further purification. A small amount of the product was further purified by CC on silica (eluent: petroleum ether:EtOAc = 2:1) and characterized. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27-1.39 (m, 6H), 1.50-1.68 (m, 4H), 2.32 (t, 2H, J= 7.5 Hz), 3.62 (t, 2H, J= 6.5 Hz), 6.87 (br. s., 2H).

[0154] Methyl 8-(tetrahydro-2*H*-pyran-2-yloxy)octanoate (503). 8-Hydroxyoctanoic acid (**502**; 26.3 g, 164 mmol) was dissolved in methanol (500 ml) containing

acetyl chloride (3.5 ml). The reaction mixture was refluxed for 5 h and the solvent removed *in vacuo*. To the residue dissolved in CH_2Cl_2 (200 ml), 3,4-dihydro-2*H*-pyran (29 ml, 318 mmol) was added, and the reaction mixture was refluxed for 20 min. Upon addition of 5 ml of triethylamine, the solvent was removed *in vacuo*, and the residue was dissolved in petroleum ether (100 ml) and washed with water. The organic layer was flush-purified on a small silica column (silica, 100 ml; eluent: from petroleum ether to petroleum ether:EtOAc = 20:1). The work-up yielded 38.2 g (90%) of the product which was used without further purification. A small amount of the product was further purified by CC on silica (eluent: petroleum ether: EtOAc = 15:1) and characterized. IR (CCl_4): $\tilde{\nu}$ = 1741 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.20-1.36 (m, 6H), 1.40-1.82 (m, 10H), 2.23 (t, 2H, J= 7.5 Hz), 3.30 (dt, 1 H, J = 9.5 Hz, 6.5 Hz), 3.39-3.46 (m, 1H), 3.59 (s, 3H), 3.65 (dt, 1 H, J = 9.5 Hz, 7.0 Hz), 3.76-3.83 (m, 1H), 4.47-4.52 (m, 1H).

[0155] **[1,1-D_2]-8-(tetrahydro-2*H*-pyran-2-yloxy)octan-1-ol (504)**. To a stirred solution of ester **(503)** (37.5 g, 145 mmol) in diethyl ether (100 ml) in an ice bath, a suspension of $LiAlD_4$ (4.0 g, 95 mmol) in diethyl ether (300 ml) was added drop wise over 1 h. To the cold reaction mixture, water (4 ml), 15% NaOH (4 ml) and water (12 ml) were added with stirring. The precipitate was filtered and washed with ethyl ether. Evaporation *in vacuo* gave 33.5 g (99%) of the product. A small amount of the product was further purified by CC on silica (eluent: petroleum ether: EtOAc = 10:1) and characterized. IR (CCl_4): $\tilde{\nu}$ = 3638, 3499 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.22-1.33 (m, 8H), 1.42-1.56 (m, 8H), 1.61-1.69 (m, 1H), 1.71-1.80 (m, 1H), 2.38 (br. s., 1H), 3.31 (dt, 1 H, J = 9.5 Hz, 6.5 Hz), 3.40-3.46 (m, 1H), 3.66 (dt, 1 H, J = 9.5 Hz, 7.0 Hz), 3.76-3.84 (m, 1H), 4.49-4.53 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.5, 25.3, 25.5, 26.0, 29.2, 29.3, 29.5, 30.6, 32.4, 62.1, 67.5, 98.7.

[0156] **[1,1-D_2]-8-(tetrahydro-2*H*-pyran-2-yloxy)octyl methanesulfonate (505)**. To a solution of alcohol **(504)** (33.4 g, 144 mmol) and triethylamine (45 ml, 323 mmol) in diethyl ether (300 ml) at 0°C, a solution of MsCl (14.2 ml, 183 mmol) in diethyl ether (100 ml) was added drop wise over 1 h with stirring. The reaction mixture was warmed up to r.t. and treated with water. The organic phase, combined with washings (2 x 50 ml) of the aqueous phase with Et_2O, was washed twice with saturated NaCl, dried over Na_2SO_4, and decanted. This was flush-purified on a small silica column (silica, 100 ml; petroleum ether:EtOAc = 10:1). The work-up yielded 43.7 g (98%) of methanesulfonate **(505)**. IR (CCl_4): $\tilde{\nu}$ = 1739 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.26-1.41 (m, 8H), 1.44-1.59 (m, 6H), 1.63-1.84 (m, 4H), 2.97 (s, 3H), 3.32 (dt, 1 H, J = 9.5 Hz, 6.5 Hz), 3.42-3.50 (m,1H), 3.69 (dt, 1 H, J = 9.5 Hz, 7.0 Hz) 3.78-3.86

(m, 1H), 4.52-4.56 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.6, 25.2, 25.4, 26.0, 28.7, 28.8, 29.1, 29.5, 30.7, 37.2, 62.3, 67.4, 98.8.

[0157] **2-([8,8-D_2]-dec-9-yne-1-yloxy)tetrahydro-2*H*-pyran (506).** Methanesulfonate (**505**) (43.5 g, 140 mmol) in DMSO (100 ml) was added dropwise with stirring over 1 h to a suspension of a ethylenediamine - lithium acetylenide complex (70 g, 0.76 mol) in DMSO (200 ml), and then the mixture was stirred for 90 min. Reaction mixture was poured on ice, extracted (Et_2O, 3 x 150 ml), dried over Na_2SO_4 and evaporated. This was flush-purified on a small silica column (silica, 100 ml; petroleum ether). Removal of solvent (rotavap) gave 25.3 g (75%) of the product. A small amount of the product was further purified by CC on silica (eluent: petroleum ether: EtOAc = 25:1) and characterized. IR (CCl_4): $\tilde{\nu}$ = 3314 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21-1.38 (m, 8H), 1.42-1.57 (m, 8H), 1.62-1.70 (m, 1H), 1.73-1.83 (m, 1H), 1.89 (s, 1H), 3.32 (d.t., 1 H, J = 9.5 Hz, 6.5 Hz), 3.42-3.50 (m, 1H), 3.68 (d.t., 1 H, J = 9.5 Hz, 7.0 Hz) 3.78-3.86 (m, 1H), 4.51-4.54 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.6, 25.4, 26.1, 28.1, 28.5, 28.9, 29.2, 29.6, 30.6, 30.7, 62.1, 67.5, 68.0, 98.7.

[0158] **[8,8-D_2]-dec-9-yne-1-ol (507).** Ether (**506**) (25 g, 104 mmol) was dissolved in methanol (300 ml) containing pyridinium *para*-toluenesulfonate (0.2 g). Reaction mixture was refluxed for 3 h, quenched with Et_3N (1 ml), the solvent removed *in vacuo*, the residue dissolved in petroleum ether and filtered through a small amount of silica gel. The solvent was evaporated to give 15.4 g (95 %) of the product. A small amount of the product was further purified by CC on silica (eluent: petroleum ether: EtOAc = 15:1) and characterized. IR (CCl_4): $\tilde{\nu}$ = 3638, 3508, 3314 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22-1.40 (m, 8H), 1.42-1.56 (m, 4H), 1.91 (s, 1H), 2.29 (br. s., 1H), 3.59 (t, J= 6.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.6, 28.1, 28.5, 29.0, 29.2, 32.6, 62.8, 68.1, 84.6.

[0159] **[8,8-D_2]-methyl dec-9-ynoate (508).** To a solution of chromium trioxide (24 g, 0.24 mol) and concentrated sulfuric acid (21 ml) in water (100 ml) in a two-neck round bottom flask on water bath at 30°C with stirring, a solution of alcohol (**507**) (15.5 g, 99 mmol) in acetone (150 ml) was added dropwise over 90 min. Upon addition, the reaction mixture was stirred for another 15 min, and the excess of oxidizer was quenched with isopropyl alcohol. The mixture was poured into cold water and extracted with diethyl ether (5 x 50 ml). Combined organic fractions were washed with saturated NaCl, dried over Na_2SO_4, filtered, and the solvent removed *in vacuo*. The residue was dissolved in methanol (200 ml) and upon addition of concentrated sulfuric acid (1 ml) refluxed for 90 min. The acid was quenched with triethylamine (6.5 ml, 47 mmol), the solvent removed *in vacuo*, and the residue purified by CC on silica (eluent: petroleum ether: EtOAc = 50:1) to give 12.6 g (69 % counting per alcohol (**507**)) of

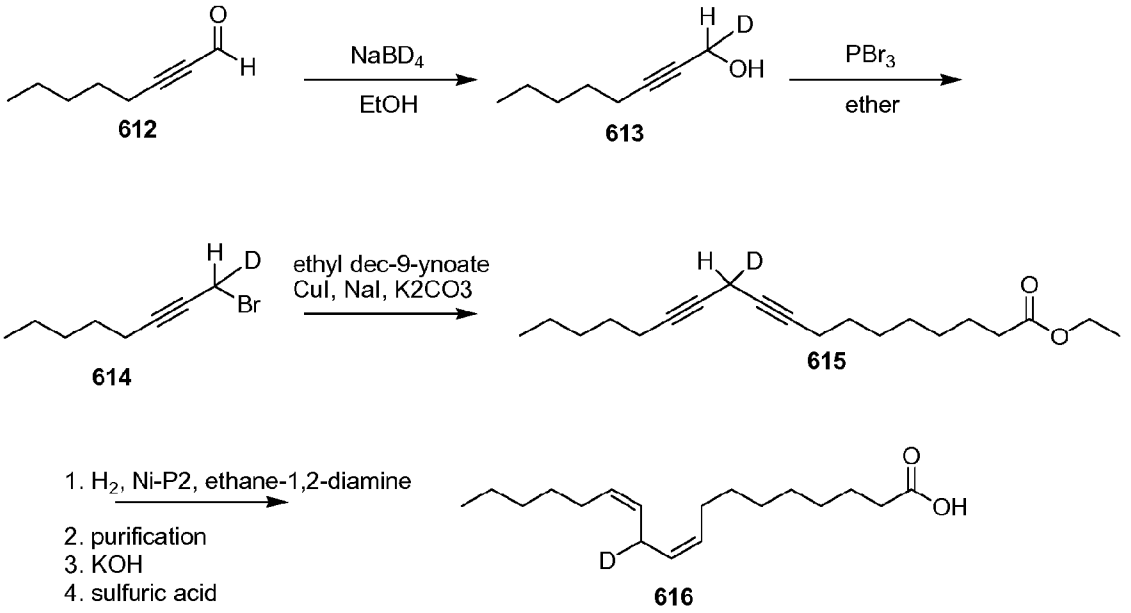
ester (**508**). and characterized. IR (CCl_4): $\tilde{\nu}$ = 3314, 1740 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.19-1.38 (m, 6H), 1.41-1.48 (m, 2H), 1.51-1.61 (m, 2H), 1.88 (s, 1H), 2.25 (t, J= 7.5 Hz, 2H), 3.60 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.7, 28.0, 28.3, 28.6, 28.8, 33.9, 51.3, 68.1, 84.4, 174.0.

[0160] **[8,8-D_2]-methyl octadeca-9,12-diynoate** (**510**). To DMF (20 ml) were added with stirring CuI (3.9 g, 20 mmol), followed by NaI (3.1 g, 21 mmol), K_2CO_3 (4.2 g, 30 mmol), ester (**508**) (1.9 g, 10.3 mmol), and bromide (**509**) (2.04 g, 10.8 mmol, synthesized as described in [2]). The reaction mixture was stirred at r.t. for 12 h. Saturated aqueous ammonium chloride (20 ml) was added to the mixture, followed by saturated NaCl (15 ml). The precipitate and the aqueous phase were washed with petroleum ether. The combined organic fractions were washed with saturated sodium chloride, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by CC on silica (eluent: petroleum ether: EtOAc = 50:1) to give 2.47 g (82 %) of the product. ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, J= 7.0 Hz, 3H), 1.22-1.36 (m, 10H), 1.40-1.50 (m, 4H), 1.55-1.64 (m, 2H), 2.09-2.15 (m, 2H), 2.28 (t, J = 7.5 Hz, 2H), 3.09 (t, J=2.5 Hz, 2H), 3.64 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.6, 13.9, 18.6, 22.1, 24.8, 28.3, 28.4, 28.5, 28.7, 28.9, 31.0, 34.0, 51.4, 74.4, 74.5, 80.2, 80.4, 174.2.

[0161] **[8,8-D_2]-octadeca-9,12-dienoate** (**511**). A suspension of finely ground $Ni(Ac)_2$ x $4H_2O$ (0.8 g, 3.2 mmol) in 96 % ethanol (25 ml) was heated with stirring to 50-60°C until the salt was fully dissolved. The system was flushed with hydrogen, and then a solution of $NaBH_4$ (3.4 ml; obtained by 15 min stirring of $NaBH_4$ suspension (0.53 g, 14 mmol) in ethanol (12 ml) followed by filtering through a fine filter) was added over 10 min. Evolvement of hydrogen was observed. In 15-20 min, ethylenediamine (1.65 ml, 25 mmol) was added to the reaction mixture in one portion with stirring, followed by the solution of (**510**) (2.4 g, 8.2 mmol) in ethanol (10 ml). The reaction mixture was vigorously stirred under hydrogen until there was no further absorption of hydrogen, and then treated with acetic acid (2.3 ml), water (10 ml), and extracted with petroleum ether:EtOAc (5:1). Combined organic fractions were washed with 10 % sulfuric acid (10 ml), then with saturated sodium chloride, dried over Na_2SO_4, and the solvent was removed *in vacuo*. The residue was purified by CC on silica (eluent: petroleum ether: EtOAc = 50:1) to give 2.33 g (96 %) of the product. The product was then purified again by CC on silica impregnated with 20 % $AgNO_3$ (eluent: petroleum ether to petroleum ether: EtOAc = 2:1). 1.75 g (72 %) of the product was obtained (97 % purity by GC). ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J= 7.0 Hz, 3H), 1.20-1.40 (m, 14H), 1.55-1.66 (m, 2H), 1.97-2.09 (m, 2H), 2.29 (t, J= 7.5 Hz, 2H), 2.72-2.79 (m, 2H), 3.66 (s, 3H), 5.28-5.41 (m, 4H). ^{13}C NMR (100 MHz,

$CDCl_3$) δ 14.0, 22.5, 24.9, 25.6, 27.2, 29.00, 29.08, 29.13, 29.3, 29.4, 31.5, 34.1, 51.4, 127.9, 128.0, 129.9, 130.2, 174.2.

Example 6. Synthesis of 11-D-Linoleic Acid



[0162] **oct-2-yn-1-ol (13).** To a solution of oct-2-ynal [*See* Corey, E.J.; Schmidt, G. *Tetrahedron Lett.* **1979**, *20*, 399; Meyer, M. P.; Klinman, J. P. *Tetrahedron Lett.* **2008**, *49*, 3600] ((**612**); 1.00 g, 8.1 mmol)) in ethanol (15 ml) cooled to 0°C, 0.11 g (2.6 mmol) of $NaBD_4$ was added in portions over 5 min. Upon addition, the solution was stirred for another 30 min, diluted with water (100 ml), and then extracted with Et_2O (4 x 20 ml). The combined organic fractions were washed with saturated NaCl, dried (Na_2SO_4), and the solvent was removed at reduced pressure. Alcohol **613** (0.85 g, 83%) was purified by column chromatography (silica gel, petroleum ether:EtOAc (15:1)). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 3H, CH_3), 1.32 (m, 4H, CH_2), 1.49 (quint, J = 7.0 Hz, 2H, CH_2), 1.81 (br s, 1H, OH), 2.19 (td, J = 7.0 Hz, 2.0 Hz, 2H, CH_2), 4.22 (m, 1H, CHD).

[0163] **1-bromooct-2-yne (614)** was synthesized as described in [*See* Hill, Sh.; Hirano, K.; Shmanai, V. V.; Marbois, B. N.; Vidovic, D.; Bekish, A. V.; Kay, B.; Tse, V.; Fine, J.; Clarkc, C. F.; Shchcpinov, M. S. *Free Radic. Biol. Med.*, **2011**, *50 (1)*, 130-138.]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, J = 7.0 Hz, 3H, CH_3), 1.32 (m, 4H, CH_2), 1.50 (quint, J = 7.0 Hz, 2H, CH_2), 2.22 (td, J = 7.0 Hz, 2.0 Hz, 2H, CH_2), 3.91 (m, 1H, CHD).

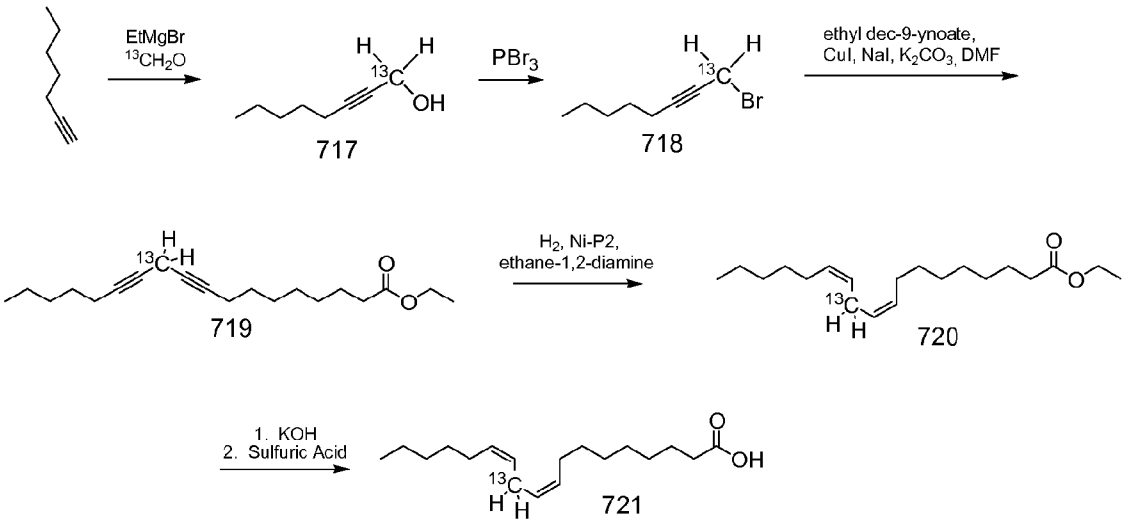
[0164] **[11-^{2}H]-ethyl octadeca-9,12-diynoate (615)**. was synthesized as described [*See* Meyer, M. P.; Klinman, J. P. *Tetrahedron Lett.* **2008**, *49*, 3600; Hill, Sh.; Hirano, K.; Shmanai, V. V.; Marbois, B. N.; Vidovic, D.; Bekish, A. V.; Kay, B.; Tse, V.; Fine, J.; Clarke, C. F.; Shchepinov, M. S. *Free Radic. Biol. Med.*, **2011**, *50 (1)*, 130-138]. CuI (2 g, 10.5 mmol),

NaI (1.58 g, 10.5 mmol), K_2CO_3 (2.1 g, 15 mmol), ethyl dec-9-ynoate (1.02 g, 5.2 mmol) and bromide **614** (1.03 g, 5.4 mmol) were added to DMF (10 ml) with stirring. The reaction mixture was stirred at RT for 12 h, then NH_4Cl (10 ml) and NaCl (8 ml) were added and the stirring continued for another 5 min. The precipitate was separated and washed with petroleum ether. Organic layers were separated, and the aqueous layer was extracted with petroleum ether. The combined organic fractions were washed with saturated NaCl, dried (Na_2SO_4), and the solvent was removed at reduced pressure. Column chromatography (silica gel, petroleum ether:EtOAc (15:1)) yielded 1.29 g (81 %) of the product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, J= 7.0 Hz, 3H, CH_3), 1.25 (t, J = 7.0 Hz, 3H, CH_3CH_2O), 1.31 (m, 10H, CH_2), 1.49 (m, 4H, CH_2), 1.61 (m, 2H, CH_2), 2.15 (td, J = 7.0 Hz, 2.0 Hz, 2H, CH_2 in propargylic position), 2.28 (t, J = 7.5 Hz, 2H, CH_2COOEt), 3.10 (m, 1H, CHD), 4.12 (q, J = 7.0 Hz, 2H, OCH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.6 (t, J = 19.0 Hz), 13.9, 14.1, 18.56, 18.57, 22.1, 24.8, 28.4, 28.6, 28.7, 28.9, 28.9, 31.0, 34.2, 60.0, 74.3, 74.5, 80.2, 80.3, 173.7.

[0165] **[11-^{2}H]-linoleic acid (616)** A suspension of triturated nickel acetate tetrahydrate (0.4 g, 1.6 mmol) in 96% ethanol (12 ml) was heated at 50-60°C with stirring until the salt dissolved. The system was flushed with hydrogen, and then 1.7 ml of $NaBH_4$ (obtained by 15-min stirring of a $NaBH_4$ suspension (0.27 g, 14 mmol) in ethanol (6 ml) followed by sfiltering) was added over 10 min, with some gas bubbles evolving. In 15-20 min, ethylenediamine (0.8 ml, 12 mmol) was added in one portion with stirring, followed in 5 min by a solution of diyne **615** (1.2 g, 3.9 mmol) in ethanol (5 ml). The reaction mixture was stirred vigorously until there was no more absorption of hydrogen, and then treated with acetic acid (1.2 ml), water (10 ml) and extracted with a mixture of petroleum ether and EtOAc (5:1). The combined organic fractions were washed with 10% sulphuric acid (5 ml) and then with saturated NaCl, dried (Na_2SO_4), and the solvent was removed at reduced pressure. Column chromatography (silica gel, petroleum ether:EtOAc (50:1)) yielded 1.14 g (94 %) of the product. The product was additionally purified [3] on a silver nitrate-impregnated silica (20% $AgNO_3$), with petroleum ether:EtOAc (2:1) as eluent to give 0.73 g (60 %) of the linoleic acid ethyl ester (> 96% purity by GC; GC-MS: MW 309 (GC-MS for a control non-deuterated linoleic acid ethyl ester: MW 308). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, J= 7.0 Hz, 3H, CH_3), 1.25 (t, J = 7.0 Hz, 3H, CH_3CH_2O), 1.30 (m, 14H, CH_2), 1.61 (m, 2H, CH_2), 2.04 (m, 2H), 2.28 (t, J= 7.5 Hz, 2H, CH_2COOEt), 2.74 (m, 1H, CHD), 4.12 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 5.34 (m, 4H, CH=CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 14.2, 22.6, 25.0, 25.3 (t, J = 19.5 Hz), 27.17, 27.19, 29.08, 29.09, 29.14, 29.3, 29.6, 31.5, 34.4, 60.1, 127.8, 128.0, 130.0, 130.2, 173.9.

[0166] To obtain the free [11-^{2}H]-linoleic acid (**616**), to the solution of the linoleic acid ethyl ester (0.704 g, 2.3 mmol) in ethanol (10 ml) a solution of KOH (0.4 g, 7.1 mmol) in water (0.8 ml) was added. The mixture was stirred at 50°C for 10 min and then diluted with water (20 ml), treated with 10 % solution of sulphuric acid (5 ml) and extracted with Et_2O (4 x 20 ml). The combined organic fractions were washed with saturated NaCl, dried over Na_2SO_4, and the solvent was removed at reduced pressure. The residue was flushed through a small volume of silica gel (2 ml; eluent: petroleum ether:EtOAc (2:1)) and the solvent removed *in vacuo* to yield 0.629 g (98 %) of the indicated acid **616**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 7.0 Hz, 3H, CH_3), 1.30 (m, 14H, CH_2), 1.60 (m, 2H, CH_2), 2.03 (m, 4H, CH_2), 2.33 (t, J = 7.5 Hz, 2H, CH_2COOEt), 2.74 (m, 1H, CHD), 5.32 (m, 4H, CH=CH), 11.6 (br s, 1H, COOH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 24.6, 25.3 (t, J = 19.0 Hz), 27.16, 27.18, 29.00, 29.05, 29.12, 29.3, 29.6, 31.5, 34.0, 127.8, 128.0, 130.0, 130.2, 180.1.

Example 7. Synthesis of [11-^{13}C]-Linoleic Acid



[0167] **[1-^{13}C]-oct-2-yn-1-ol (717).** The title compound has been synthesizedaccording to the earlier described protocols (Hill, Sh.; Hirano, K.; Shmanai, V. V.; Marbois, B. N.; Vidovic, D.; Bekish, A. V.; Kay, B.; Tse, V.; Fine, J.; Clarke, C. F.; Shchepinov, M. S. *Free Radic. Biol. Med.*, **2011**, *50 (1)*, 130-138) using ^{13}C-paraform, and used without further purification. ^{1}H NMR ($CDCl_3$, δ): 4.22 (д, J = 148 Hz, 2H), 2.18 (td, J_1 = 7.0, J_2 = 1 Hz, 2H), 1.91 (br s, 1H), 1.47 (quint, J = 7.0 Hz, 2H), 1.31 (m, 4H), 0.87 (t, J = 7.0 Hz, 3H).

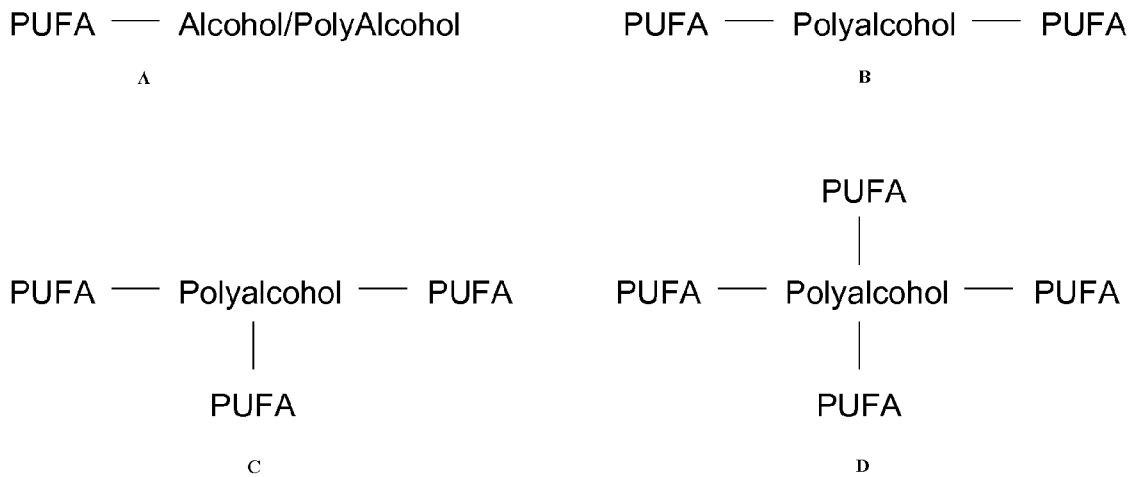
[0168] **[1-^{13}C]-1-bromooct-2-yne (718)** was synthesized as described in (Hill, Sh.; Hirano, K.; Shmanai, V. V.; Marbois, B. N.; Vidovic, D.; Bekish, A. V.; Kay, B.; Tse, V.; Fine, J.; Clarke, C. F.; Shchepinov, M. S. *Free Radic. Biol. Med.*, **2011**, *50 (1)*, 130-138). Yield: 82% starting from ^{13}C-paraform (per two steps). ^{1}H NMR ($CDCl_3$, δ): 3.93 (dt, J_1 = 158 Hz, J_2 = 2Hz, 2.23 (m, 2H), 1.50 (m, 2H), 1.33 (m, 4H), 0.89 (t, J = 7 Hz, 3H).

[0169] **[11-^{13}C]-ethyl octadeca-9,12-diynoate (719)**. was synthesized as previously described (*See* Meyer, M. P.; Klinman, J. P. *Tetrahedron Lett.* **2008**, *49*, 3600; Hill, Sh.; Hirano, K.; Shmanai, V. V.; Marbois, B. N.; Vidovic, D.; Bekish, A. V.; Kay, B.; Tse, V.; Fine, J.; Clarke, C. F.; Shchepinov, M. S. *Free Radic. Biol. Med.*, **2011**, *50 (1)*, 130-138). Yield: 93%. ^{1}H NMR ($CDCl_3$, δ): 4.10 (q, J = 7 Hz, 2H), 3.1 (dm, J = 134 Hz, 2H), 2.27 (t, J = 7.5 Hz, 2H), 2.13 (m, 4H), 1.60 (m, 2H), 1.47 (m, 4H), 1.3 (m, 10H), 1.24 (t, J = 7 Hz, 3H), 0.88 (t, J = 7.0 Hz, 3H).

[0170] **[11-^{13}C]-linoleic acid ethyl ester (720)** was synthesized as previously described (*See* Meyer, M. P.; Klinman, J. P. *Tetrahedron Lett.* **2008**, *49*, 3600; Hill, Sh.; Hirano, K.; Shmanai, V. V.; Marbois, B. N.; Vidovic, D.; Bekish, A. V.; Kay, B.; Tse, V.; Fine, J.; Clarke, C. F.; Shchepinov, M. S. *Free Radic. Biol. Med.*, **2011**, *50 (1)*, 130-138). Yield: 56%. ^{1}H NMR ($CDCl_3$, δ): 5.34 (m, 4H), 4.12 (q, J = 7 Hz, 2H), 2.77 (dm, J = 126 Hz, 2H), 2.28 (t, J = 7.5 Hz, 2H), 2.04 (m, 4H), 1.61 (m, 2H), 1.30 (m, 14H), 1.25 (t, J = 7 Hz, 3H), 0.88 (t, J = 7.0 Hz, 3H).

[0171] **[11-^{13}C]-linoleic acid (721)** was synthesized as previously described (*See* Meyer, M. P.; Klinman, J. P. *Tetrahedron Lett.* **2008**, *49*, 3600; Hill, Sh.; Hirano, K.; Shmanai, V. V.; Marbois, B. N.; Vidovic, D.; Bekish, A. V.; Kay, B.; Tse, V.; Fine, J.; Clarke, C. F.; Shchepinov, M. S. *Free Radic. Biol. Med.*, **2011**, *50 (1)*, 130-138); yield 98%. ^{1}H NMR ($CDCl_3$, δ): 10.5 (br s, 1H), 5.34 (m, 4H), 2.77 (dm, J = 126 Hz), 2.33 (t, J = 7.5 Hz, 2H), 2.03 (m, 4H), 1.60 (m, 2H), 1.30 (m, 14H), 0.88 (t, J = 7.0 Hz, 3H).

Example 8. General Preparation of Esters A-D



[0172] **General Procedure for Compound A.** Thionyl chloride (2 equivalents) is slowly added to a solution of PUFA (1 equivalent) in $CHCl_3$. The reaction mixture is heated to reflux for 1 hr, then it is allowed to cool to room temperature and the solvent is evaporated under reduced pressure to afford the carboxylic acid chloride derivative of the PUFA. The

carboxylic acid chloride derivative is then dissolved in anhydrous pyridine and the alcohol (1 equivalent) dissolved in pyridine is slowly added (Note that the order of addition is reversed when the alcohol is a polyalcohol). Upon complete addition, the reaction mixture is allowed to stir at room temperature for 24 hr. The solvent is then removed under reduced pressure and the crude product is purified by column chromatography to afford Compound A.

[0173] 11,11-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**30**); 14,14-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**23**); 11,11,14,14-Tetradeutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**17**); and 11,11-Dideutero-cis,cis-octadeca-9,12-dienoic acid (**7**) are each subjected to the above described procedure with the following alcohols: ethanol, glycerol, propylene glycol; glucose; 2-(2-ethoxyethoxy)ethanol; and estradiol to afford products corresponding to the general formula of Compound A.

[0174] **General Procedure for Compound B.** Thionyl chloride (2 equivalents) is slowly added to a solution of PUFA (1 equivalent) in $CHCl_3$. The reaction mixture is heated to reflux for 1 hr, then it is allowed to cool to room temperature and the solvent is evaporated under reduced pressure to afford the carboxylic acid chloride derivative of the PUFA. The carboxylic acid chloride derivative is then dissolved in anhydrous pyridine and the alcohol (Compound A, 1 equivalent) dissolved in pyridine is slowly added. Upon complete addition, the reaction mixture is allowed to stir at room temperature for 24 hr. The solvent is then removed under reduced pressure and the crude product is purified by column chromatography to afford Compound B.

[0175] The Compound A products that form from the condensation of 11,11-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**30**); 14,14-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**23**); 11,11,14,14-Tetradeutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**17**); and 11,11-Dideutero-cis,cis-octadeca-9,12-dienoic acid (**7**) with glycerol, propylene glycol; glucose; and estradiol are treated according to the above-described general procedure with 11,11-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**30**); 14,14-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**23**); 11,11,14,14-Tetradeutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**17**); and 11,11-Dideutero-cis,cis-octadeca-9,12-dienoic acid (**7**) as the PUFAs to afford products corresponding to the general formula of Compound B.

[0176] **General Procedure for Compound C.** Thionyl chloride (2 equivalents) is slowly added to a solution of PUFA (1 equivalent) in $CHCl_3$. The reaction mixture is heated to reflux for 1 hr, then it is allowed to cool to room temperature and the solvent is evaporated under reduced pressure to afford the carboxylic acid chloride derivative of the PUFA. The carboxylic acid chloride derivative is then dissolved in anhydrous pyridine and the alcohol

(Compound B, 1 equivalent) dissolved in pyridine is slowly added. Upon complete addition, the reaction mixture is allowed to stir at room temperature for 24 hr. The solvent is then removed under reduced pressure and the crude product is purified by column chromatography to afford Compound C.

[0177] The Compound B products that form from the condensation of Compound A products with glycerol and glucose are treated according to the above-described general procedure with 11,11-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**30**); 14,14-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**23**); 11,11,14,14-Tetradeutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**17**); and 11,11-Dideutero-cis,cis-octadeca-9,12-dienoic acid (**7**) as the PUFAs to afford products corresponding to the general formula of Compound C.

[0178] **General Procedure for Compound D.** Thionyl chloride (2 equivalents) is slowly added to a solution of PUFA (1 equivalent) in $CHCl_3$. The reaction mixture is heated to reflux for 1 hr, then it is allowed to cool to room temperature and the solvent is evaporated under reduced pressure to afford the carboxylic acid chloride derivative of the PUFA. The carboxylic acid chloride derivative (4 equivalents) is then dissolved in anhydrous pyridine and the alcohol (1 equivalent) dissolved in pyridine is slowly added. Upon complete addition, the reaction mixture is allowed to stir at room temperature for 24 hr. The solvent is then removed under reduced pressure and the crude product is purified by column chromatography to afford Compound D.

[0179] The Compound C products that form from the condensation of Compound B products with glucose are treated according to the above-described general procedure with 11,11-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**30**); 14,14-Dideutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**23**); 11,11,14,14-Tetradeutero-cis,cis,cis-octadeca-8,12,15-trienoic acid (**17**); and 11,11-Dideutero-cis,cis-octadeca-9,12-dienoic acid (**7**) as the PUFAs to afford products corresponding to the general formula of Compound D.

Example 9. ^{1}H- and ^{13}C-NMR analysis of deuterated PUFAs described in Examples 1-4 (Figure 2).

[0180] Characteristic areas of ^{1}H and ^{13}C spectra, all values in ppm. (*Panel A*) Deuteration of Lin acid at pos. 11 is confirmed by the disappearance of peaks in ^{1}H and ^{13}C NMR spectra. Disappearance of the peak at δ_H 2.764 is expected due to absence of H atoms (^{1}H NMR). Disappearance of the peak at δ_C 25.5 in is due to combination of Nuclear Overhauser Effect, and splitting of this particular carbon atom into a quintet by two D atoms in the deuterated form of Lin acid. (*Panel B*) The ^{1}H NMR spectrum shows that the H atoms at C11 and C14 positions of site-specifically deuterated αLnn coincide (δ_H 2.801) thus deuteration at

either site (11,11-H_2, 14,14-D_2 or 11,11-D_2, 14,14-H_2) leads to a 50% decrease in integration of this peak, while deuteration of both sites (11,11,14,14-D_4) leads to the complete disappearance of the peak at δ_H 2.801. However, ^{13}C NMR experiments can clearly distinguish between the three deuterated forms, as the observed peaks for C11 and C14 positions are separated by a small but detectable difference. Thus, deuteration at either C11 or C14 positions leads to disappearance of the peak at δ_C 25.68 or δ_C 25.60, respectively, while deuteration at both sites leads to disappearance of the two corresponding peaks.

Example 10. Isotope Reinforcement Can Shut Down PUFA peroxidation

[0181] Q-less yeast (coq mutants) provide an ideal system to assess in vivo autoxidation of fatty acids. Coenzyme Q (ubiquinone or Q) serves as a small lipophilic antioxidant as well as an electron shuttle in the respiratory chain of the mitochondrial inner membrane. Ten S. cerevisiae genes (COQ1-COQ10) are required for coenzyme Q biosynthesis and function, and the deletion of any results in respiratory deficiency (Tran UC, Clarke CF. *Mitochondrion* **2007**;*7S*,S62). It was shown that the coq yeast mutants are exquisitely sensitive to autoxidation products of PUFAs (Do TQ et al, *PNAS USA* **1996**;*93*:7534-7539; Poon WW, Do TQ, Marbois BN, Clarke CF. *Mol. Aspects Med.* **1997**;*18*,s121). Although S. cerevisiae do not produce PUFAs (Paltauf F, Daum G. *Meth. Enzymol.* **1992**;*209*:514-522), they are able to utilize PUFAs when provided exogenously, allowing their content to be manipulated (Paltauf F, Daum G. *Meth. Enzymol.* **1992**;*209*:514-522). Less than 1% of Q-less (coq2, coq3, and coq5) yeast mutants is viable following a four hour treatment with linolenic acid (Do TQ et al, *PNAS USA* **1996**;*93*:7534-7539; Poon WW, Do TQ, Marbois BN, Clarke CF. *Mol. Aspects Med.* **1997**;*18*,s121). In contrast, 70% of wild-type (the parental genetic background is strain W303-1B) cells subjected to this treatment remain viable. The Q-less yeast are also hypersensitive to other PUFAs that readily autoxidize (such as arachidonic acid), but behave the same as the wild-type parental strain to treatment with the monounsaturated oleic acid (Do TQ et al, *PNAS USA* **1996**;*93*:7534-7539). The hypersensitivity of the Q-less yeast mutants is not a secondary effect of the inability to respire, because cor1 or atp2 mutant yeast (lacking either the bc1 complex or the ATP synthase, respectively) show wild-type resistance to PUFA treatment (Do TQ et al, *PNAS USA* **1996**;*93*:7534-7539; Poon WW, Do TQ, Marbois BN, Clarke CF. *Mol. Aspects Med.* **1997**;*18*,s121).

[0182] A plate dilution assay can be used to assess PUFA sensitivity. This assay can be performed by spotting serial five-fold dilutions of aliquots onto YPD plate media (**Fig. 3**). The sensitivity of the different strains can be observed by visual inspection of the density of cells in each spot.

[0183] Treatment with linolenic acid causes a dramatic loss of viability of the *coq* null mutants. In stark contrast, *coq* mutants treated with the D4-linolenic acid were not killed, and retained viabilities similar to yeast treated with oleic acid. Quantitative colony counting revealed that the viability of cells treated with oleic and D4-linolenic was similar (**Fig. 4**), while the viability of the *coq* mutants was reduced more than 100-fold following treatment with the standard linolenic acid for 4h. These results indicate that isotope-reinforced linolenic acid is much more resistant to autoxidation than is the standard linolenic acid, as evidenced by the resistance of the hypersensitive coq mutants to cell killing.

Example 11. GC-MS Can Detect Fatty Acids and PUFAs in Yeast Cells

[0184] Yeast do not synthesize PUFAs, however they do incorporate exogenously supplied linoleic and linolenic acids (Avery SV, et al. *Applied Environ. Microbiol.* **1996**; *62*,3960; Howlett NG, et al. *Applied Environ. Microbiol.* **1997**; *63*,2971). Therefore, it seems likely that yeast would also incorporate exogenously supplied D4-linolenic acid. However, it is possible that the differential sensitivity to linolenic and D4-linolenic might be attributed to differences in integration into the cell rather than autoxidation. To test whether this is the case, the extent of uptake of this fatty acid was monitored. First the conditions of separation of fatty acid methyl esters (FAME) of C18:1, C18:3, D4-18:3 and C17:0 (to be used as an internal standard) were determined. The GC-MS chromatogram shown in **Fig. 5** establishes both separation and sensitivity of detection of these fatty acid methyl ester standards.

[0185] Wild-type yeast were harvested during log phase growth and incubated in the presence of exogenously added fatty acid (for 0 or 4 h) in the presence of phosphate buffer plus 0.20% dextrose, as described for the fatty acid sensitivity assay. Cells were harvested, washed twice with 10 ml sterile water, and the yeast cell pellets were then processed by alkaline methanolysis as described above. The fatty acids are detected as methylesters (FAMEs) following GC-MS with C17:0 added as an internal standard (**Fig. 6**). The amounts of 18:3 and D4 detected after 4 h incubation were extrapolated from the calibration curve. These results indicate yeast avidly incorporate both linolenic and D4-linolenic acid during the 4 h incubation period. Based on these results, it is obvious that the enhanced resistance of the *coq* mutant yeast to treatment with D4-C18:3 is not due to lack of uptake.

[0186] D2-linolenic, 11, 11-D2-linolenic acid and 14, 14-D2-linolenic acid, were also used on this yeast model and rendered comparable protection.

Example 12. Kinetic isotope effect in non-enzymatic oxidation of D2-LA in a chain reaction format.

[0187] The kinetics of oxygen consumption during the oxidation of LA and D2-LA was studied with a glass capillary microvolumeter (Figure 7). The rate of oxidation, R_{OX}, was measured as a slope of $[O_2]$ traces. The rate of initiation, R_{IN}, was determined by the inhibitor method with HPMC ("6-hydroxy-2,2,5,7,8-pentamethylbenzochroman") as a reference inhibitor. R_{IN} was calculated from the induction period of inhibited oxidation, t_{IND}: $R_{IN} = 2 \cdot [HPMC]/t_{IND}$. The rate of oxidation of 0.71 M LA (Fig. 7) was found to be 6.1×10^{-6} M/s. When the process was inhibited by 0.23 mM chain-breaking antioxidant HPMC, the duration of the induction period, t_{IND}, was about 48 min, with the R_{IN} value of around 0.16×10^{-6} M/s. The length of the kinetic chain calculated from these data was: $\nu = R_{OX}/R_{IN} = 38 \pm 3$. Based on this data, the calculated oxidizability of LA was 0.0215 ± 0.008 $M^{-0.5}s^{-0.5}$ (n = 5) [Cosgrave J.P, et. al. *Lipids*, **1987**, *22*, 299-304]. For D2-LA, the reduction of R_{OX} to 0.18×10^{-6} M/s was observed (Figure 7). In contrast to LA, addition of HPMC did not result in the decrease in R_{OX} and the appearance of any detectable induction period (data not shown). The latter precludes a direct determination of R_{IN}. For a R_{IN} value for D2-LA oxidation being comparable to that of LA it follows that D2-LA oxidation was not a chain process ($\nu = 0.18 \times 10^{-6}/0.16 \times 10^{-6} \approx 1.1$). An estimated kinetic isotope effect ("KIE"), from comparison of R_{OX} for LA and D2-LA, was around $6.1 \times 10^{-6}/0.18 \times 10^{-6} \approx 35$. A similar KIE was determined during the oxidation of LA and 11,11-d_2-LA in Triton X-100 aqueous micelles (data not shown). For comparative purposes, the theoretical KIE is 6.9 at 25° C. *See* Carpenter, "Determination of Organic Reaction Mechanisms" (John Wiley & Sons, 1984), p. 89.

Example 13. Small Amounts of D2-LA Protect LA Against Peroxidation.

[0188] To simulate the likely *in vivo* conditions, the kinetics of the oxidation of the mixtures of D2-LA and LA were studied (Figure 8). In the experiments, the concentration of LA plus 11,11-d2-LA was 0.775 M; the concentration of AMVN was 0.0217 M; and the reactions were carried out at 37° C. The results afforded an R_{IN} of $1.10 \pm 0.08 \times 10^{-7}$ M/sec. Additionally, the rate of oxidation of the mixtures was found to be non-additive and much lower than the additive value of R_{OX} for the individual compounds. Surprisingly, D2-LA essentially 'protects' the non-deuterated LA against autoxidation. A qualititatively similar effect was also observed during the oxidation of the mixture of 11,11-D2-LA with non-deuterated methyl linoleate (data not shown). These results suggest that even a partial replacement of non-deuterated LA by D2-LA may substantially slow down PUFA peroxidation.

Example 14. Small amounts of D2-LA Protect LA Against Peroxidation *In Vivo*.

[0189] The results described in Example 13 were reproduced *in vivo* using Q-less *coq3* yeast strains and different ratios of LA to D2-LA (Figure 9). Wild-type, yeast Q-less *coq3,* or respiratory deficient *cor1* null mutants were incubated in the presence of 200 μM of LA and D2-LA at different ratios of PUFAs, as indicated in Fig. 9. Serial dilutions (1:5) starting at 0.2OD/ml were spotted on YPD solid plate medium. Additionally, a zero-time untreated control was utilized and the results are shown on the top left of Fig. 9. Growth was at 30° C. The results indicate that approximately 10-15% of D2-LA was a sufficiently minimal amount to cancel the toxicity of LA. A similar incubation with the mono-deuterated PUFA, 11,11-D,H-LA, afforded no detectable loss in cell viability after 3 hours of treatment (data not shown). These results suggest that both D2-LA and 11,11-D,H-LA were resistant to lipid peroxidation.

[0190] Wild-type yeast cells were treated as described above except the yeast were treated with 200 μM of the designated fatty acid for 2 hours, washed with sterile water, and were either not treated (triangles) or treated with 50 μM $CuSO_4$ (squares) at room temperature. After 60 min of copper treatment cells were treated with 8 μM C11-Bodipy 581/591 for 30 min at room temperature. Four 100 μl aliquots were plated in a 96-well plate and the fluorescence was measured. Wild-type yeast cells treated with copper in the absence or presence of PUFA have significantly higher levels of lipid peroxidation as compared to yeast not treated with copper. However, copper-stressed wild-type yeast cells treated with 11,11-D_2-LA have lower levels of lipid peroxidation similar to yeast not treated with PUFA. Mono-deuterated 11,11-D,H-LA offered similar protection.

Example 15. Small Amounts of D4-ALA Protect ALA Against Peroxidation *In Vivo*.

[0191] The experimental protocol described for Example 14 was also reproduced *in vivo* using Q-less *coq3* yeast strains (Figure 10) and different ratios of ALA to D4-ALA. Wild-type, yeast Q-less *coq3,* or respiratory deficient *cor1* null mutants were incubated in the presence of 200 μM of ALA and D4-Lnn (Linolenic acid) at different ratios of PUFAs, as indicated in Fig. 10. Serial dilutions (1:5) starting at 0.2OD/ml were spotted on YPD solid plate medium. Growth was at 30° C. The results indicate that approximately 15-20% of D2-Lnn was a sufficiently minimal amount to cancel the toxicity of ALA. Moreover, results indicate that the content of PUFA taken up by yeast cells roughly reflects the ratios added and suggests that yeast cells do not discriminate among the PUFAs provided.

Example 16. D-PUFA mitigates oxidative stress and increases survival in retinal cells implicated in AMD and Diabetic Retinopathy pathology

[0192] Several cell types, including microvascular endothelium (MVEC), retinal pigment epithelium (RPE) and retinal neurons (retinal ganglion cells) were tested for survival in cell culture. Cells were kept in the medium containing either hydrogenated (control) or deuterated D2-linoleic (ω-6; LA) and D4-linolenic (ω-3; ALA) acids (20 μM; ratio of ω-6 to ω-3: 1:1 or 2:1) for 72 hrs. The incorporation of PUFAs into cells was monitored by GC (**Figure 11**). PUFAs were shown to be readily taken up by cells according to the Table 1, showing incorporation of PUFAs into MVECs.

Table 1

		Area unlabelled	**Area labelled**	**ratio**
control	linoleate	78392976	4556042	0.058
	linolenate	1488866	149411	0.100
PUFA	linoleate	96026830	5525295	0.058
	linolenate	2347729	113468	0.048
Deuterated PUFA	linoleate	34957060	2599969	0.074
	linolenate	747128	134824	0.180

[0193] The cells were then treated with paraquat (PQ; 500 μM), a common oxidative stress-generating compound. For survival measurement, cells were counted using haemocytometer and trypan blue exclusion method. **Figure 12** shows the survival of H- and D-PUFA treated MVEC cells after acute intoxication by paraquat. For all cell types tested, D-PUFA had protective effect compared to controls, similar to that shown in Figure 8 for MVEC cells.

Example 17. Toxicology studies of mice supplemented with D-PUFA reveal no anomalies in major blood biomarkers.

[0194] With a more protracted dosing paradigm (i.e. 3 weeks of dietary replacement), chemical analysis of blood serum of H-PUFA- and D-PUFA-supplemented mice (performed at UC Davis) revealed no difference in major biomarkers of renal function, liver function, blood lipids, etc for H-PUFA/D-PUFA saline treated mice. In this example, D-PUFA is a 2:1 mixture of D2-linoleic acid: D4-linolenic acid.

[0195] Tested parameters included measurements of triglycerides; total protein; total bilirubin; phosphorus; free fatty acids; HDL; glucose; creatine; cholesterol; calcium; blood urea nitrogen; alkaline phosphatase; albumin; aspartate aminotransferase; and others in Table 2.

Table 2

Mouse ID #	Sample volume	Alanine Aminotransferase U/L	Aspartate Aminotransferase U/L	Albumin g/dl	Alkaline Phosphatase U/L	Blood Urea Nitrogen mg/dl	Calcium mg/dl	Cholesterol mg/dl	Creatinine mg/dl	Glucose mg/dl	High Density Lipoprotein mg/dl	Non-esterified Fatty Acid mEq/L	Phosphorus mg/dl	Total Bilirubin mg/dl	Total Protein g/dl	Triglyceride mg/dl
4	100	273.0	3008.7	3.09	81.7	19.1	7.96	148.3	0.189	160.2	104.49	1.08	13.07	0.185	5.32	38.9
5	110	5726.7	8478.9	3.42	31.1	25.4	7.40	185.1	0.356	355.6	134.37	1.07	18.59	0.275	6.56	57.9
7	100	156.0	1470.6	2.82	35.1	18.9	7.64	151.2	0.154	174.6	107.39	1.11	10.14	0.192	5.26	82.7
10	60	518.4	4653.0	3.02	QNS	20.1	6.78	184.0	0.151	136.5	138.15	1.06	QNS	0.272	6.07	46.1
11	70	144.0	1635.3	3.63	72.7	20.3	8.75	170.8	0.179	107.9	139.86	1.18	9.33	0.162	5.72	33.5
13	14	3518.1	15669.0	QNS	<0.1	31.5	QNS	166.5	1.126	176.4	135.09	0.99	QNS	QNS	QNS	31.5
14	75	216.9	2107.8	3.03	42.4	24.4	7.46	173.6	0.170	93.3	47.78	1.06	10.41	0.235	6.07	43.8
25	75	589.5	4707.0	3.20	18.8	18.0	5.97	193.4	0.126	164.5	147.96	1.01	18.39	0.269	6.74	41.0
27	100	727.2	6015.6	2.63	<0.1	36.2	5.71	166.7	1.453	88.3	98.46	0.87	24.57	0.301	6.26	26.9
28	100	468.9	4018.5	2.93	49.3	21.2	6.90	164.4	0.232	224.9	50.54	1.02	14.16	0.231	5.87	49.6
29	29	1896.1	12510.0	QNS	QNS	24.9	QNS	208.6	0.111	QNS	77.58	0.20	QNS	QNS	QNS	27.9
30	100	2963.7	5371.2	3.38	50.3	18.2	6.29	174.7	0.225	227.4	131.04	1.17	21.42	0.349	6.28	46.7
Mean D-PUFA	76	1508	5289	3.17	52.6	22.8	7.67	168.5	0.332	172.1	115.30	1.08	12.31	0.220	5.83	47.8
SD D-PUFA	33	2225	5189	0.30	23.0	4.6	0.66	14.5	0.357	87.0	33.21	0.06	3.78	0.048	0.50	17.7
Mean H-PUFA	81	1329	6524	3.04	39.5	23.7	6.22	181.6	0.429	176.3	101.12	0.85	19.64	0.268	6.29	38
SD D-PUFA	31	1078	3428	0.33	17.9	8	0.51	19.0	0.575	65.5	39.40	0.38	4.44	0.050	0.36	11

Example 18. Histopathologic Studies

[0196] Microscopic changes were coded by the most specific topographic and morphologic diagnosis, and the Systematized Nomenclature of Medicine (SNOMED) and the National Toxicology Program's Toxicology Data Management System (TDMS) terminology manuals were used as guidelines. Data were recorded in Labcat® Histopathology module 4.30. A four-step grading system (minimal, mild, moderate, and marked) was used to define gradable changes.

[0197] C57BL6 male mice were dosed orally in the diet with PUFAs on Study Days 1 through 14, and were necropsied on Study Day 15. Group 1 consisted of 4 mice and received hydrogenated PUFAs. Group 2 consisted of 5 mice and received deuterated PUFAs (D2-LA and D4-ALA) On Study Day 8, all mice received intraperitoneal (IP) saline. Complete sets of

protocol-specified tissues [liver (3 to 7 sections), lungs with bronchi (2 to 5 lobes), spleen, heart, and kidneys] from all submitted mice were examined histopathologically. No difference was observed between the H-PUFA and D-PUFA groups.

Example 19. Evaluation of Tissue-specific Deuteration

[0198] WT mice were housed at 12 animals (males separate from females) per cage and fed for 90 days ad libitum (typically, 5-6 g/day) on the AIN 93 diet, as pellets, with 6% total fat. Approximately 10% of that total fat was made up of 1:1 mixture of D2-LA/D4-ALA (group 1), D2-LA/ALA (group 2), or LA/ALA (control group). The animals were sacrificed, organs harvested and stored at low temperature prior to analysis without the use of preservation agents. Lipid fractions were separated, pre-treated and analyzed by LC-MS according to standard protocols using LA, D2-LA, ALA and D4-ALA as controls.

[0199] Dosage studies of 1:1 D2-LA/D4-ALA indicated that tissues became highly enriched in deuterium, with about 40% of the total fat being deuterated (**Fig. 13**). Moreover, these studies indicated that fat distribution remained relatively unchanged by the tested dosage (**Fig. 14**). After dosage studies of 1:1 D2-LA/ALA, it was determined that about 27% of the total fat was deuterated (**Fig. 15**).

[0200] Specific organs, such as the liver and brain, were also evaluated (**Figs. 16-21**). While the liver had a different fat profile than previous tissues studied (**Fig. 16**), 90 day dosage studies with D2-LA/D4-ALA demonstrated that tissues became highly enriched in deuterium, with about 40% of the total fat being deuterated (**Fig. 17**). Moreover, the liver study indicated that fat distribution remained relatively unchanged by the tested dosage (**Fig. 16-17**). Additionally, 90 day dosage studies with D2-LA only illustrated a similar fat profile as previous studies, along with about 32% total fat being deuterated (**Fig. 18**). Consequently, fat profiles and deuteration profiles in the liver were maintained regardless of the administered deuterated component. Like the liver, the brain also had a different fat profile than previous tissues studied (**Figs. 19-21**). 90 day dosage studies with D2-LA/D4-ALA demonstrated that tissues became highly enriched in deuterium, with about 30% of the total fat being deuterated (**Fig. 19**). Moreover, the brain study indicated that fat distribution remained relatively unchanged by the tested dosage (**Figs. 19-21**). Additionally, 90 day dosage studies with D2-LA/ALA illustrated a similar fat profile as previous studies, along with about 23% total fat being deuterated (**Fig. 20**). Consequently, fat profiles and deuteration profiles in the brain were maintained regardless of the administered deuterated component.

Example 20: Neurodegenerative Disorder Treatment

[0201] Primary embryonic hippocampal neuronal cells are widely recognized as useful in models of neuronal function. Briefly, primary cultures of hippocampal neurons can be used to test compounds for activity in neuronal protection. Hippocampal cultures can typically be prepared from 18- to 19-day fetal rats. At this age, the generation of pyramidal neurons, which begins in the rat at about E15, would be essentially complete. The brain tissue at this stage would be relatively easy to dissociate, the meninges could be readily removed, and the number of glial cells still would be relatively modest (Park L C, Calingasan N Y, Uchida K, Zhang H, Gibson G E. (2000). Metabolic impairment is known to elicit brain cell type-selective changes in oxidative stress and cell death in culture. *J Neurochem* 74(1):114-124). In order to evaluate the activity of compounds disclosed herein, a test compound could be assessed for its ability to protect cells against beta-amyloid induced oxidative stress in hippocampal neurons. For example, cultured hippocampal neurons are obtained from fetal rats and incubated with test compounds (0.01, 0.1, 1.0, 10.0, and 100 mg of D2-LA, D4-ALA, and 1:1 combinations of both D2-LA and D4-ALA) while subjected to beta-amyloid induced oxidative stress. Control cells treated with similar weights and combinations of non-deuterated LA and ALA and subjected to beta-amyloid induced oxidative stress are also studied. Comparing the two groups of cells, D2-LA and D4-ALA are expected to be efficacious at inhibiting beta-amyloid formation.

[0202] Protection against redox stress can be further evaluated in cell culture using high glutamate induced oxidative stress (HGOS) in mouse dopaminergic cell lines. The cytotoxic effect of glutamate is not due to excitotoxicity, as this cell line is devoid of inotropic glutamate receptors. Rather, the glutamate-induced toxicity of dopaminergic cells is associated with an inhibition of cystine transport which subsequently leads to depletion of intracellular glutathione (GSH) levels (Murphy T. H., et al. Neuron 2, 1547-1558, 1989), activation of neuronal 12-lipoxygenase (Li, Y. et al., *Neuron* 19,453 463, 1997), increased ROS production (Tan S. et al., *J. Cell Biol.* 141,1423-1432, 1998) and elevated intracellular Ca^{2+} (Li, Y. et al., see supra).). In order to evaluate the activity of compounds disclosed herein, a test compound could be assessed for its ability to protect cells against glutamate-induced stress. For example, mouse dopaminergic cell lines are obtained and incubated with test compounds (0.01, 0.1, 1.0, 10.0, and 100 mg of D2-LA, D4-ALA, and 1:1 combinations of both D2-LA and D4-ALA) while subjected to glutamate-induced stress. Control cells treated with similar amounts and combinations of non-deuterated LA and ALA and subjected to glutamate-induced stress are also studied. Comparing the two groups of cells, D2-LA and D4-ALA are expected to be efficacious at inhibiting glutamate-induced toxicity.

[0203] Further validation of neuroantiinflammatory activity of compounds could be assessed in vitro by the inhibition of IL-1β release from a microglial cell line. Interleukin-1 (IL-1) is a proinflammatory cytokine that exists in two separate forms that share 30% sequence homology (alpha and beta). Constitutive expression of IL-1 is low in the brain but levels of both forms of this cytokine increase dramatically after injury. There is substantial evidence that IL-1 is an important mediator of neurodegeneration induced by cerebral ischemia (Touzani O et al, *J Neuroimmunol.,* 100:203-215, (1999)). Both IL-1 forms are rapidly induced in experimental models of stroke and administration of recombinant IL-1 beta enhances ischemic injury (see Hill J K. et al. *Brain Res.* 820:45-54, (1999), Hillhouse E W et al. *Neurosci Lett* 249:177-179, (1998), Loddick S A et al *J Cereb Blood Flow Metab* 16:932-940, (1996), Stroemer R P et al., *J Cereb Blood Flow Metab.* 18:833-839, (1998)). Conversely, blocking IL-1 actions with a receptor antagonist or a neutralizing antibody markedly reduces neuronal death and inflammation in models of ischemic damage (see Betz A L, *J Cereb Blood Flow Metab* 15:547-551, (1995); Relton J K, *Brain Res Bull* 29:243-246, (1992); Yamasaki Y et al, Stroke 26:676-680, (1995)). Furthermore, mice with decreased IL-1β production (caspase-1 knockouts) are significantly protected from ischemic injury (Schielke G P, et al. *J Cereb Blood Flow Metab* 18:180-185, (1998)) and IL-1α and β double knockouts exhibit dramatically reduced ischemic infarct volumes compared with wild-type mice (87% reduction in cortex) (Boutin H et al., *J Neurosci* 21:5528-5534, (2001)).

[0204] Additionally, IL-1 elevation has been associated with many neurodegenerative diseases. There is increasing evidence for a role of IL-1 in Alzheimer's Disease (AD) (Mrak R E et al. Neurobiol Aging 22(6):903-908, (2001)). Elevated levels of IL-1β have been shown to surround amyloid plaques in the disease and recent genetic studies have indicated that a polymorphism in IL-1α is linked to an increased risk of AD (3-6 fold increase) (Griffin W S et al., J Leukoc Biol 72(2):233-238, (2002)). This polymorphism has also been correlated with rate of cognitive decline in AD patients (Murphy G M et al., Neurology, 56(11)1595-1597, (2001)). The risk of AD is increased even further when the polymorphism in IL-1α is found in combination with another polymorphism in IL-1β (see Griffin W S, supra), providing convincing evidence that these cytokines play an important role in the pathology of the disease.

[0205] An assay could be used to measure the release of IL-1β from a mouse microglial cell line following an inflammatory challenge with LPS and interferon-gamma. The ability of test compounds to inhibit microglial cell activation and IL-1β release could be determined by co-incubation of the test compound with the inflammatory challenge. For

example, mouse microglial cell lines are obtained and incubated with test compounds (0.01, 0.1, 1.0, 10.0, and 100 mg of D2-LA, D4-ALA, and 1:1 combinations of both D2-LA and D4-ALA) while LPS and interferon-gamma are used to induce an inflammatory response. Control cells treated with similar weights and combinations of non-deuterated LA and ALA and subjected to LPS and interferon-gamma are also studied. Comparing the release of IL-1β from the two groups of cells, D2-LA and D4-ALA are expected to be efficacious at inhibiting the release of IL-1β.

[0206] Further validation of efficacy in neuroprotection can be assessed in functional tests, such as the grip strength test or the rotorod test. Animals treated with compounds that show neuroprotection are expected to maintain their pre-MCAO grip strength values after MCAO, as compared to untreated animals, which should show a significant reduction in grip strength, indicating loss of sensorimotor function. Likewise, animals treated with compounds that show neuroprotection should maintain their pre-MCAO rotorod activity scores after MCAO, as compared to untreated animals, which should show a significant reduction in rotorod scores, indicating loss of sensorimotor function at higher brain levels. For example, APPswe/Ps1dE9 mice (which are a recognized mouse model of Alzheimer's disease) of are given daily various dosages (0.01, 0.1, 1.0, 10.0, and 100 mg/kg of D2-LA, D4-ALA, or 1:1 combinations of both D2-LA and D4-ALA) of test compounds. A control solution (comprising similar amounts and combinations of non-deuterated LA and ALA) is given to certain other test animals. Animals are treated for 14 weeks starting at the age of 7.5 months, each of the groups contained 15 animals; all groups were balanced for age gender. After the final treatment animals receive a lethal dose of Ketamine/Xylazine and are transcardially perfused with ice-cold PBS (pH 7.4). Brains are isolated and divided sagitally; one hemi brain is snap frozen in liquid nitrogen and stored at −80° C until further processing, the second hemisphere is post-fixed in 4% PFA in PBS, dehydrated and embedded in paraffin for histological analyses. The frozen brain halves of the animals are homogenized according to standard protocols and the $A\beta_{1-40}$ and $A\beta_{1-42}$ –levels in the are measured with the hAmyloid β40 and β42 ELISA Kits (The Genetics Company, Schlieren, Switzerland). Comparing the $A\beta_{1-40}$ and $A\beta_{1-42}$ –levels from the two groups of animals, D2-LA and D4-ALA are expected to be efficacious at inhibiting the formation of amyloid-beta.

[0207] Additionally, test compounds could be evaluated for their ability to protect memory function and oxidative stress in intracerebroventricular (ICV) administered streptozotocin (STZ) induced dementia in rats. Rats dementia is induced by injecting them with STZ (3 mg/kg, ICV). Test compounds are given daily at various dosages (0.01, 0.1, 1.0, 10.0,

and 100 mg/kg of D2-LA, D4-ALA, or 1:1 combinations of both D2-LA and D4-ALA). A control solution (comprising similar amounts and combinations of non-deuterated LA and ALA) is given to control animals. The STZ (ICV) treated group should show memory deficit as indicated by no significant decrease in latency time in Morris water maze test and significant decrease in brain insulin receptor protein level in both hippocampus and cerebral cortex. Pre- and post-treatment of test compounds in STZ (ICV) treated rats are expected to prevent and/or restore the memory deficit and brain insulin receptor protein level in both of the regions.

Example 21: Neuromuscular Disease Treatment

[0208] Glutamate is believed to induce far greater reactive oxygen species (ROS) generation in cultured motor neurons than in other spinal neurons. *See* Rao et al., The Journal of Neuroscience (2003), 23(7), 2627-2633. Additionally, ROS are believed able to migrate from motor neurons and induce oxidation and disruption of glutamate uptake in neighboring astrocytes. *Id.* Moreover, it has been demonstrated in a transgenic mouse model of ALS that protein oxidation was increased in regions immediately surrounding motor neurons. *Id.*

[0209] Compounds disclosed herein can be demonstrated to protect against oxidative damage in ALS models as described for the testing of antioxidants in Rao et al., The Journal of Neuroscience (2003), 23(7), 2627-2633. For example, cultured neurons are obtained from embryonic Swiss-Webster mice and incubated with compounds according to known methods. Spinal cord immunohistochemical studies are undertaken using SOD1 G93A transgenic mice and also represent a source of neuronal cells.

[0210] Additionally, a familial form of ALS is seen in approximately 10% of cases, with several of those cases caused by point mutations in the SOD1 gene. *See* Rakhit et al., J. Biol. Chem. (2002), 277, 47551-47556. Transgenic mice that harbor FALS-associated SOD1 mutations are known to develop ALS-like symptoms. Such mice are used as test subjects for determining a compound's ability to prevent the development of ALS-like symptoms. Furthermore, human erythrocytes having wild-type Cu-Zn SOD1 are available and zinc-deficient SODs can easily be prepared. *See* Rakhit et al., J. Biol. Chem. (2002), 277, 47551-47556, and the references cited therein. Such cell lines can be used for determining a compounds ability to prevent oxidized SOD1 formation according to the methodology described in Rakhit et al., J. Biol. Chem. (2002), 277, 47551-47556.

[0211] Compounds disclosed herein are screened in astrocytes and/or myeline-laden macrophages and the results of the screening can be used to demonstrate a compound's efficacy at preventing oxidative damage in cells implicated in MS. For example, an initial screen

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identifies PUFA compounds effective for the amelioration of redox disorders in astrocytes and/or myeline-laden macrophages. Test compounds, one or more reference compounds (e.g. Idebenone, decylubiquinone, Trolox and α-tocopherol acetate), and solvent controls are tested for their ability to rescue astrocytes and/or myeline-laden macrophages stressed by the addition of compounds known to cause oxidative damage.

[0212] Experimental autoimmune encephalomyelitis (EAE) is an inflammatory autoimmune demyelinating disease which can be induced in laboratory animals by injection of myelin basic protein. Such disease has become the standard laboratory model for studying clinical and experimental autoimmune diseases. In fact, numerous articles [e.g., Abramsky, et al., J. Neuroimmunol., 2, 1 (1982) and Bolton et al., J. Neurol. Sci., 56, 147 (1982)] note that the similarities of chronic relapsing EAE in animals to multiple sclerosis in humans especially implicates the value of EAE for the study of autoimmune demyelinating diseases such as multiple sclerosis. As such, the EAE test model can be employed to establish the activity of the compounds disclosed herein against multiple sclerosis. Such testing is conducted according to the following procedure.

[0213] Female Lewis rats are injected in their footpads with 12.5 mg of myelin basic protein (MBP) (prepared from guinea-pig spinal cord) in Complete Freunds adjuvant. PUFA test compounds are given daily from day 0 (MBP injection date) at various dosages (0.01, 0.1, 1.0, 10.0, and 100 mg/kg of D2-LA, D4-ALA, or 1:1 combinations of both D2-LA and D4-ALA) to the test animals. A control solution (comprising similar weights and combinations of non-deuterated LA and ALA) is given to certain other test animals. The animals are then weighed and scored daily for symptoms of EAE according to a scale of 0 to 3 (0=no change; 1=flaccid tail; 2=hind limb disability and 3=hind quarter paralysis/moribund). Given a daily dosing paradigm, the compounds disclosed herein are shown to inhibit the progression of EAE. As such, the compounds are expected to be efficacious in treating multiple sclerosis.

Conclusion

[0214] While the invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true scope of the invention. This includes embodiments which do not provide all of the benefits and features set forth herein. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective and scope of the present invention. All such modifications are intended to be within the scope of the invention.

of the claims appended hereto. Accordingly, the scope of the invention is defined only by reference to the appended claims.

What is claimed is:

1. A polyunsaturated substance for use in the preparation of a medicine for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester in the medicine is at least 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

2. A polyunsaturated substance for use in the preparation of a medicine for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester in the medicine is about 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

3. The polyunsaturated substance of claim 1 or 2, wherein the medicine further comprises an antioxidant or is for administration with one or more antioxidants.

4. The polyunsaturated substance of claim 3, wherein the antioxidant is Coenzyme Q, idebenone, mitoquinone, mitoquinol, vitamin C, or vitamin E.

5. A polyunsaturated substance for use for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester in the medicine is at least 5% of the total amount of polyunsaturated fatty acid or fatty acid ester

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delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

6. A polyunsaturated substance for use for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester in the medicine is about 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

7. The polyunsaturated substance of any one of claims 1 to 6, wherein the polyunsaturated substance is a deuterated polyunsaturated fatty acid.

8. The polyunsaturated substance of any one of claims 1 to 6, wherein the polyunsaturated substance is a deuterated polyunsaturated fatty acid ester.

9. The polyunsaturated substance of claim 8, wherein the deuterated polyunsaturated fatty acid ester is an alkyl ester, a triglyceride, diglyceride, or monoglyceride.

10. The polyunsaturated substance of claim 9, wherein the deuterated fatty acid ester is an ethyl ester.

11. The polyunsaturated substance of any one of claims 1 to 10, wherein at least one deuterium atom of the deuterated polyunsaturated fatty acid or fatty acid ester is present at a level greater than 0.02% of naturally occurring hydrogen.

12. The polyunsaturated substance of any one of claims 1 to 11, wherein the deuterated polyunsaturated fatty acid or fatty acid ester is from about 10% to about 50% of the total amount of polyunsaturated fatty acids or fatty acid esters delivered to the patient.

13. The polyunsaturated substance of any one of claims 1 to 12, wherein the deuterated polyunsaturated fatty acid or fatty acid ester is from about 10% to about 30% of the total amount of polyunsaturated fatty acids or fatty acid esters delivered to the patient.

14. The polyunsaturated substance of any one of claims 1 to 13, wherein the deuterated polyunsaturated fatty acid or fatty acid ester is about 20% or more of the total amount of polyunsaturated fatty acids or fatty acid esters delivered to the patient.

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15. The polyunsaturated substance of any one of claims 1 to 14, wherein the polyunsaturated substance is for a cell or tissue of the patient to maintain a sufficient concentration of the deuterated polyunsaturated fatty acid or fatty acid ester to reduce or prevent autooxidation of a naturally occurring polyunsaturated substance.

16. The polyunsaturated substance of any one of claims 1 to 15, wherein the polyunsaturated substance is an omega-3 fatty acid, an omega-3 fatty acid ester, an omega-6 fatty acid, or an omega-6 fatty acid ester.

17. The polyunsaturated substance of claim 16, wherein the polyunsaturated substance is 11,11-D2-linolenic acid, 14,14-D2-linolenic acid, 11,11,14,14-D4-linolenic acid, 11,11-D2-linoleic acid, 11-D-linolenic acid, 14-D-linolenic acid, 11,14-D2-linolenic acid, or 11-D-linoleic acid, or an ester of any one of the forgoing.

18. The polyunsaturated substance of claim 16, wherein the deuterated polyunsaturated fatty acid or fatty acid ester is arachidonic acid or an ester thereof.

19. The polyunsaturated substance of claim 16, wherein the deuterated fatty acid or fatty acid ester is eicosapentaenoic acid or an ester thereof.

20. The polyunsaturated substance of claim 16, wherein the deuterated fatty acid or fatty acid ester is docosahexaenoic acid or an ester thereof.

21. The polyunsaturated substance of claim 16, wherein the deuterated fatty acid or fatty acid ester is a linoleic acid or an ester thereof.

22. The polyunsaturated substance of claim 16, wherein the deuterated fatty acid or fatty acid ester is a linolenic acid or an ester thereof.

23. The polyunsaturated substance of any one of claims 16 to 22, wherein the polyunsaturated substance is further stabilized at a pro-bis-allylic position.

24. The polyunsaturated substance of any one of claims 1 to 6, wherein the deuterated polyunsaturated fatty acid or fatty acid ester is 11,11-D2-linoleic acid or ethyl ester thereof.

25. The polyunsaturated substance of any one of claims 1 to 6, wherein the deuterated polyunsaturated fatty acid or fatty acid ester is 11,11,14,14-D4-linolenic acid or ethyl ester thereof.

26. The polyunsaturated substance of any one of claims 1 to 25, wherein the deuterated polyunsaturated fatty acid or fatty acid ester delivered to the patient is in the range of 0.1 mg/kg to 100 mg/kg per day.

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27. The polyunsaturated substance of any one of claims 1 to 25, wherein the deuterated polyunsaturated fatty acid or fatty acid ester delivered to the patient is about 0.1 mg/kg per day.

28. Use of a polyunsaturated substance for use in the preparation of a medicine for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester in the medicine is at least 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

29. Use of a polyunsaturated substance for use in the preparation of a medicine for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester in the medicine is about 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

30. The use of claim 28 or 29, wherein the medicine further comprises an antioxidant or is for administration with one or more antioxidants.

31. The use of claim 30, wherein the antioxidant is Coenzyme Q, idebenone, mitoquinone, mitoquinol, vitamin C, or vitamin E.

32. Use of a polyunsaturated substance for use for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester is at least 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the

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patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

33. Use of a polyunsaturated substance for use for treating or preventing progression of: (a) Alzheimer's Disease, Mild Cognitive Impairment, or Frontotemperal Dementia; or (b) Amyotrophic Lateral Sclerosis or Multiple Sclerosis in a patient; wherein, the polyunsaturated substance is a polyunsaturated fatty acid or fatty acid ester deuterated at one or more bis-allylic positions; and wherein the amount of deuterated polyunsaturated fatty acid or fatty acid ester is about 5% of the total amount of polyunsaturated fatty acid or fatty acid ester delivered to the patient; and wherein the deuterated polyunsaturated fatty acid or fatty acid ester is for incorporation into the patient following administration.

34. The use of any one of claims 28 to 33, wherein the polyunsaturated substance is a deuterated polyunsaturated fatty acid.

35. The use of any one of claims 28 to 33, wherein the polyunsaturated substance is a deuterated polyunsaturated fatty acid ester.

36. The use of any one of claims 28 to 35, wherein at least one deuterium atom of the deuterated polyunsaturated fatty acid or fatty acid ester is present at a level greater than 0.02% of naturally occurring hydrogen.

37. The use of any one of claims 28 to 36, wherein the deuterated polyunsaturated fatty acid or fatty acid ester is from about 10% to about 50% of the total amount of polyunsaturated fatty acids or fatty acid esters delivered to the patient.

38. The use of any one of claims 28 to 37, wherein the deuterated polyunsaturated fatty acid or fatty acid ester is from about 10% to about 30% of the total amount of polyunsaturated fatty acids or fatty acid esters delivered to the patient.

39. The use of any one of claims 28 to 38, wherein the deuterated polyunsaturated fatty acid or fatty acid ester is about 20% or more of the total amount of polyunsaturated fatty acids or fatty acid esters delivered to the patient.

40. The use of any one of claims 28 to 39, wherein the polyunsaturated substance is for a cell or tissue of the patient to maintain a sufficient concentration of the deuterated polyunsaturated fatty acid or fatty acid ester to reduce or prevent autooxidation of a naturally occurring polyunsaturated substance.

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41. The use of any one of claims 28 to 40, wherein the polyunsaturated substance is an omega-3 fatty acid, an omega-3 fatty acid ester, an omega-6 fatty acid, or an omega-6 fatty acid ester.

42. The use of claim 41, wherein the polyunsaturated substance is 11,11-D2-linolenic acid, 14,14-D2-linolenic acid, 11,11,14,14-D4-linolenic acid, 11,11-D2-linoleic acid, 11-D-linolenic acid, 14-D-linolenic acid, 11,14-D2-linolenic acid, or 11-D-linoleic acid, or an ester of any one of the forgoing.

43. The use of claim 41, wherein the deuterated polyunsaturated fatty acid or fatty acid ester is arachidonic acid or an ester thereof.

44. The use of claim 41, wherein the deuterated fatty acid or fatty acid ester is eicosapentaenoic acid or an ester thereof.

45. The use of claim 41, wherein the deuterated fatty acid or fatty acid ester is docosahexaenoic acid or an ester thereof.

46. The use of claim 41, wherein the deuterated fatty acid or fatty acid ester is a linoleic acid or an ester thereof.

47. The use of claim 41, wherein the deuterated fatty acid or fatty acid ester is a linolenic acid or an ester thereof.

48. The use of any one of claims 41 to 47, wherein the polyunsaturated substance is further stabilized at a pro-bis-allylic position.

49. The use of any one of claims 28 to 48, wherein the deuterated polyunsaturated fatty acid ester is an alkyl ester, a triglyceride, diglyceride, or monoglyceride.

50. The use of claim 48, wherein the deuterated fatty acid ester is an ethyl ester.

51. The use of any one of claims 28 to 33, wherein the deuterated polyunsaturated fatty acid or fatty acid ester is 11,11-D2-linoleic acid or ethyl ester thereof.

52. The use of any one of claims 28 to 33, wherein the deuterated polyunsaturated fatty acid or fatty acid ester is 11,11,14,14-D4-linolenic acid or ethyl ester thereof.

53. The use of any one of claims 28 to 52, wherein the deuterated polyunsaturated fatty acid or fatty acid ester delivered to the patient is in the range of 0.1 mg/kg to 100 mg/kg per day.

54. The use of any one of claims 28 to 52, wherein the deuterated polyunsaturated fatty acid or fatty acid ester delivered to the patient is about 0.1 mg/kg per day.

Date Reçue/Date Received 2020-09-14

FIG. 1A

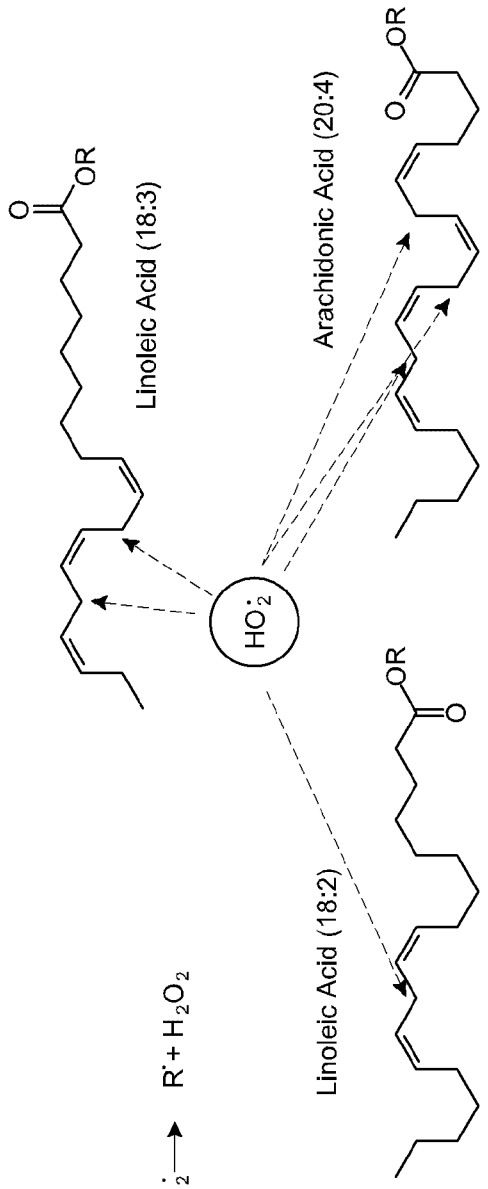
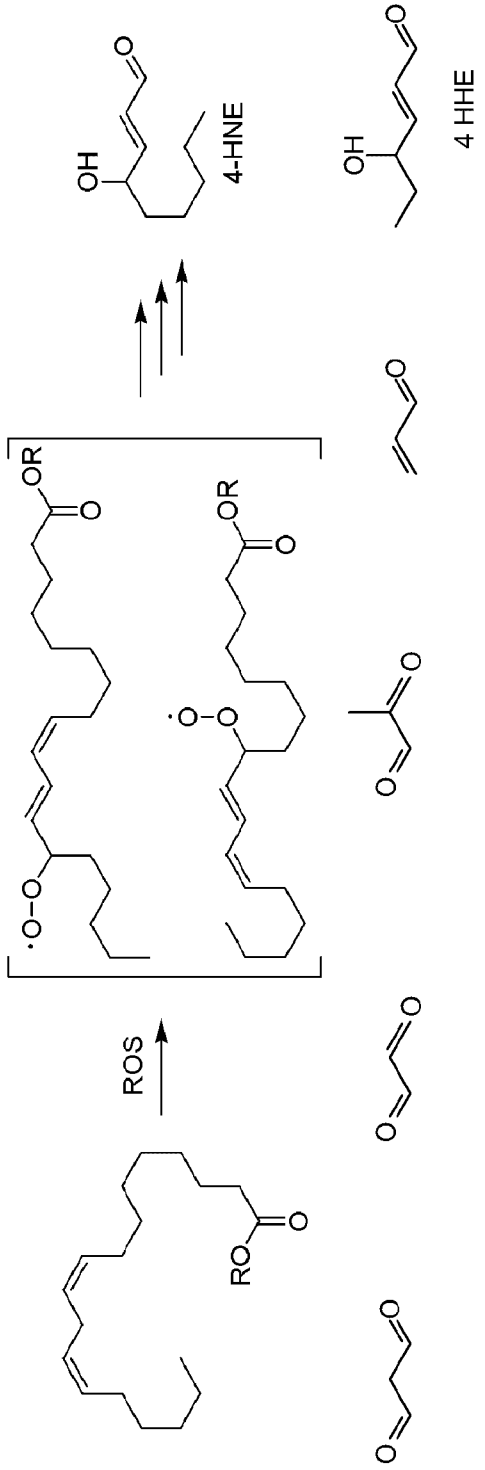

R-H + HO2· → R· + H2O2
Linoleic Acid (18:3)
Arachidonic Acid (20:4)
Linoleic Acid (18:2)
HO2·
OR
O

FIG. 1B


ROS
RO
OR
·O–O
OH
4-HNE
4 HHE

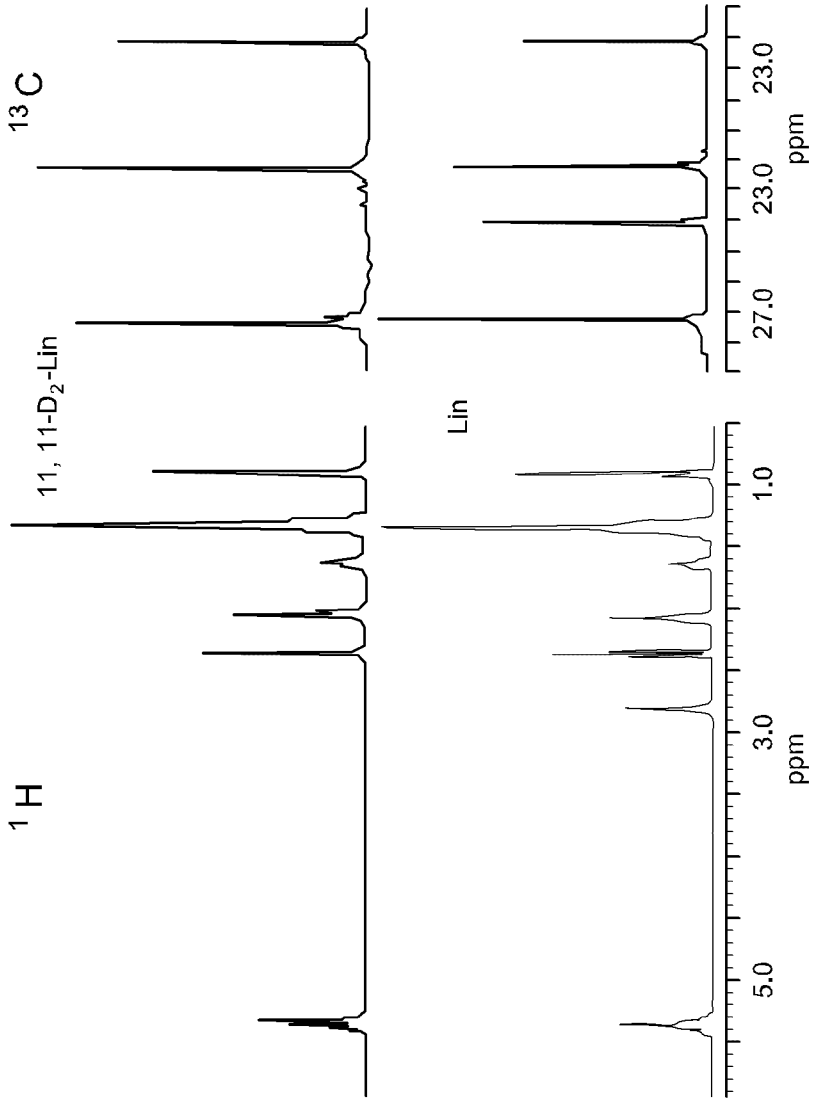


FIG. 2A

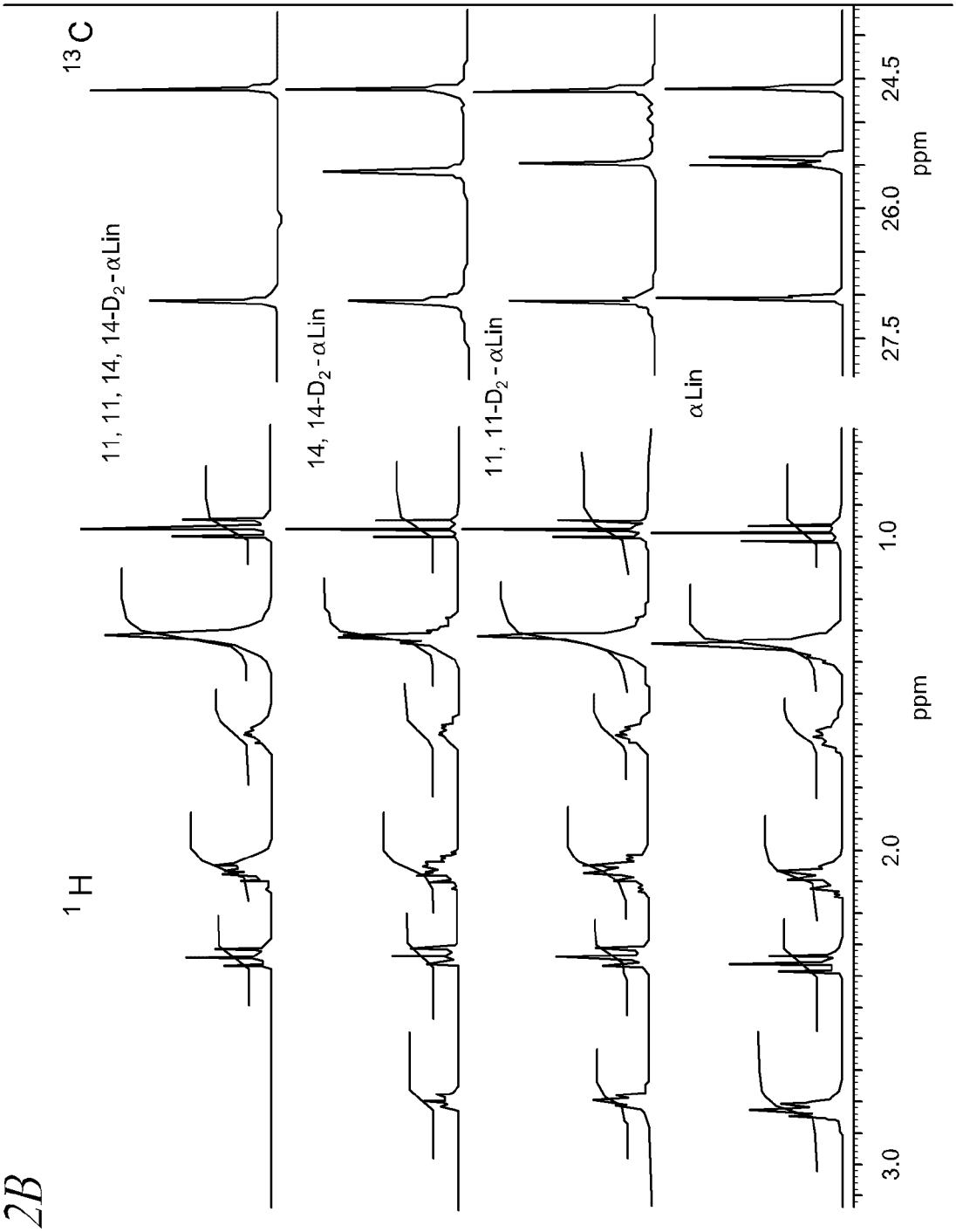

^{13}C
11, 11, 14, 14, 14-D_2-αLin
14, 14-D_2-αLin
11, 11-D_2-αLin
αLin
27.5
26.0
24.5
ppm
^{1}H
3.0
2.0
1.0
ppm

FIG. 2B

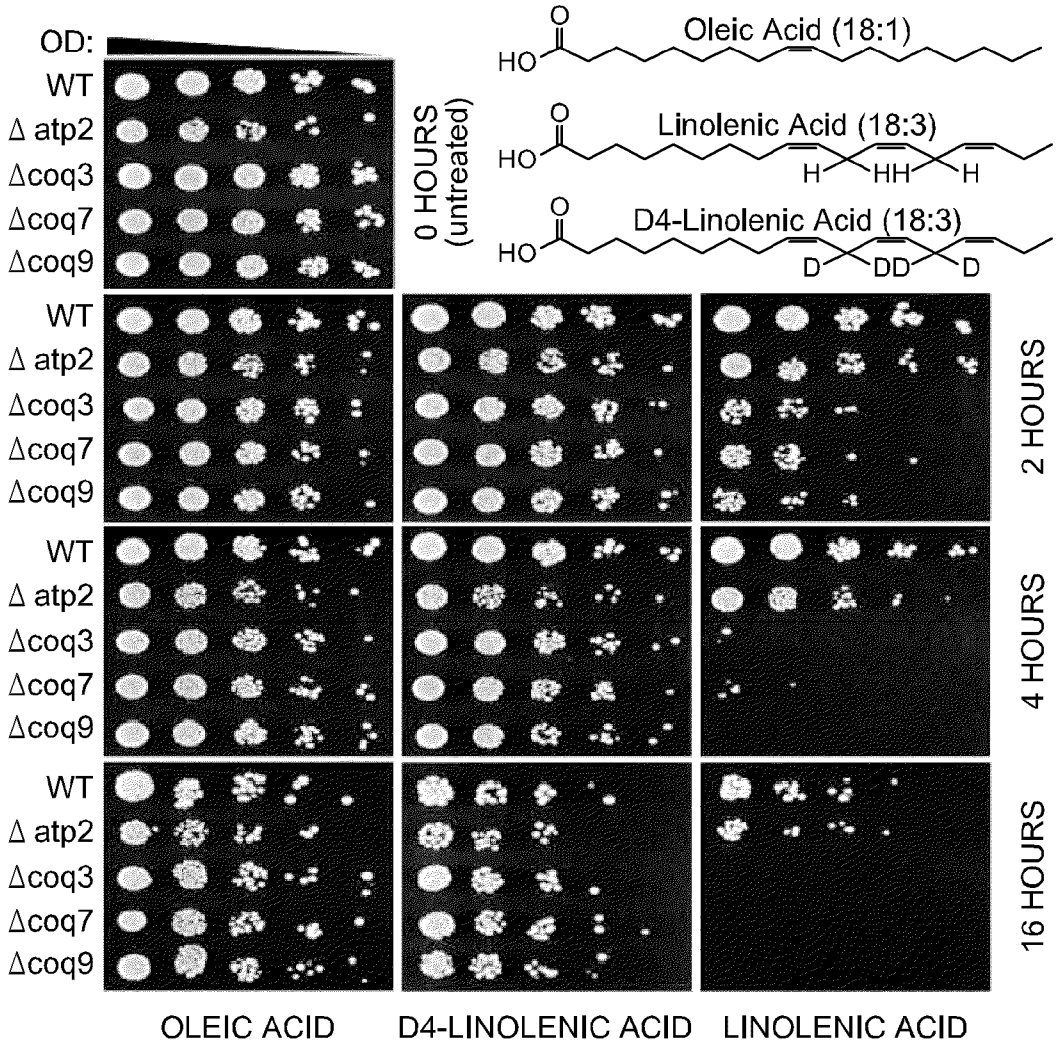

OD:
WT
Δ atp2
Δcoq3
Δcoq7
Δcoq9
0 HOURS (untreated)
Oleic Acid (18:1)
Linolenic Acid (18:3)
D4-Linolenic Acid (18:3)
2 HOURS
4 HOURS
16 HOURS
OLEIC ACID
D4-LINOLENIC ACID
LINOLENIC ACID

FIG. 3

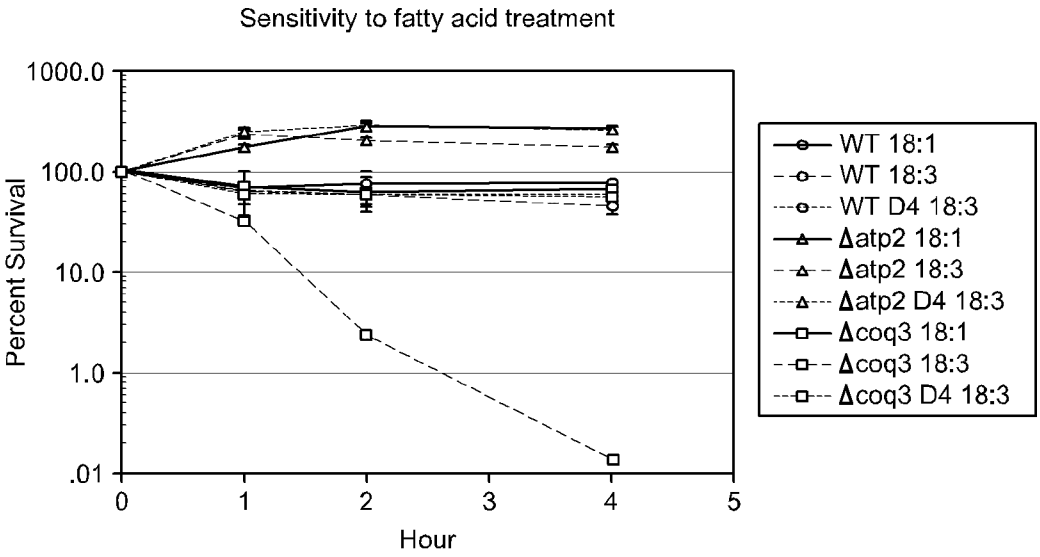

Sensitivity to fatty acid treatment
1000.0
100.0
10.0
1.0
.01
Percent Survival
0
1
2
3
4
5
Hour
WT 18:1
WT 18:3
WT D4 18:3
Δatp2 18:1
Δatp2 18:3
Δatp2 D4 18:3
Δcoq3 18:1
Δcoq3 18:3
Δcoq3 D4 18:3

FIG. 4

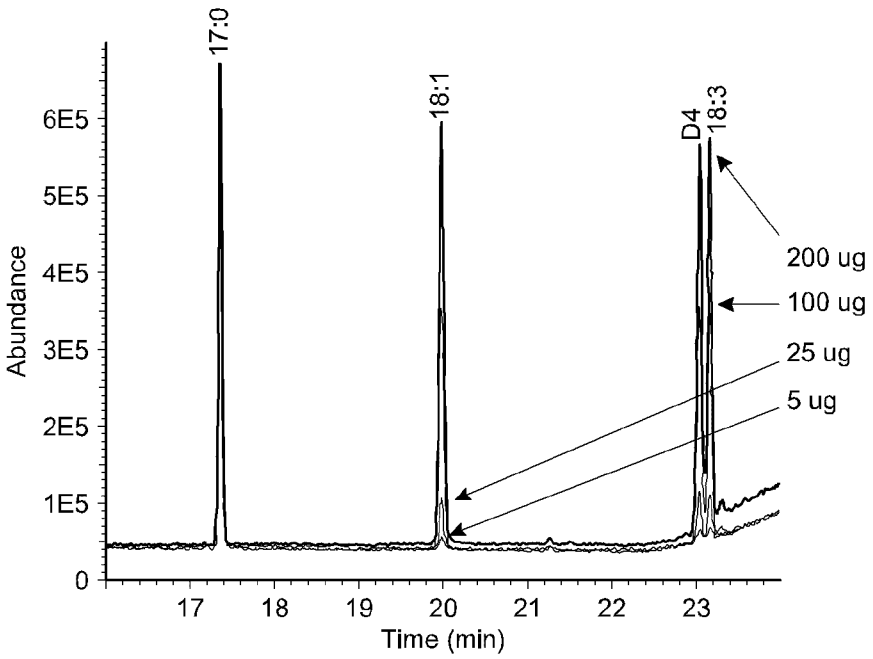

17:0
18:1
D4
18:3
200 ug
100 ug
25 ug
5 ug
Abundance
6E5
5E5
4E5
3E5
2E5
1E5
0
17
18
19
20
21
22
23
Time (min)

FIG. 5

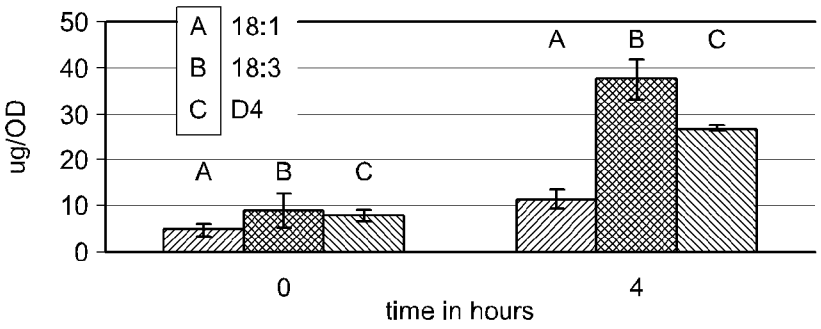


FIG. 6

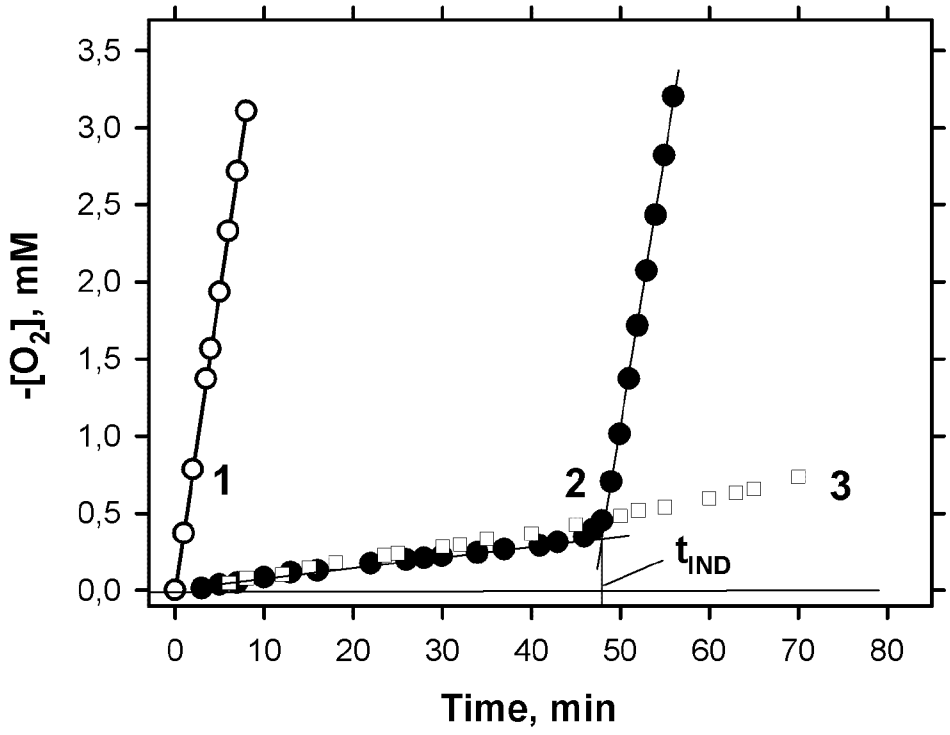

3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
-[O2], mM
0
10
20
30
40
50
60
70
80
Time, min
1
2
3
tIND

FIG. 7

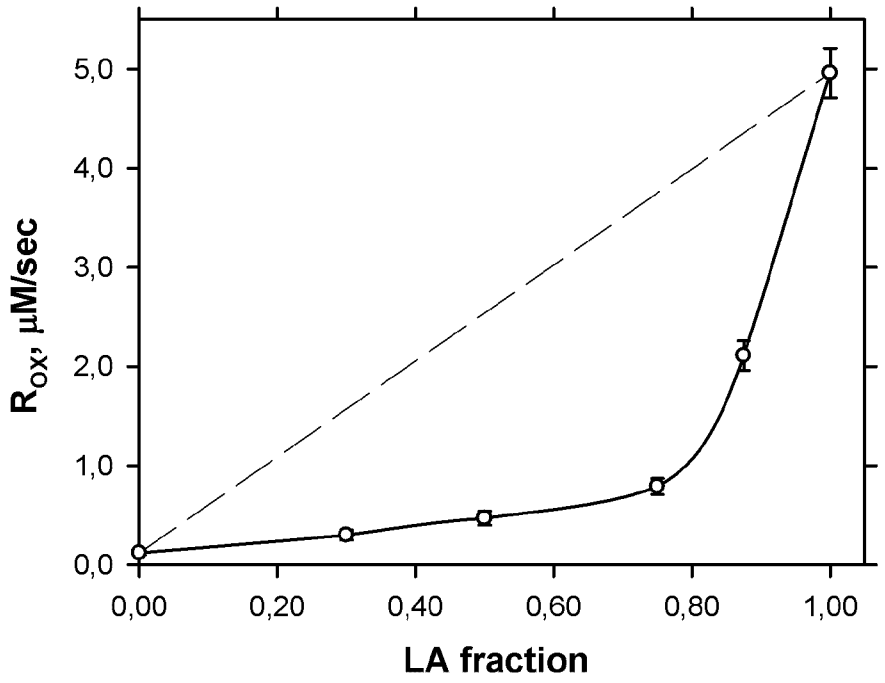


FIG. 8

FIG. 9

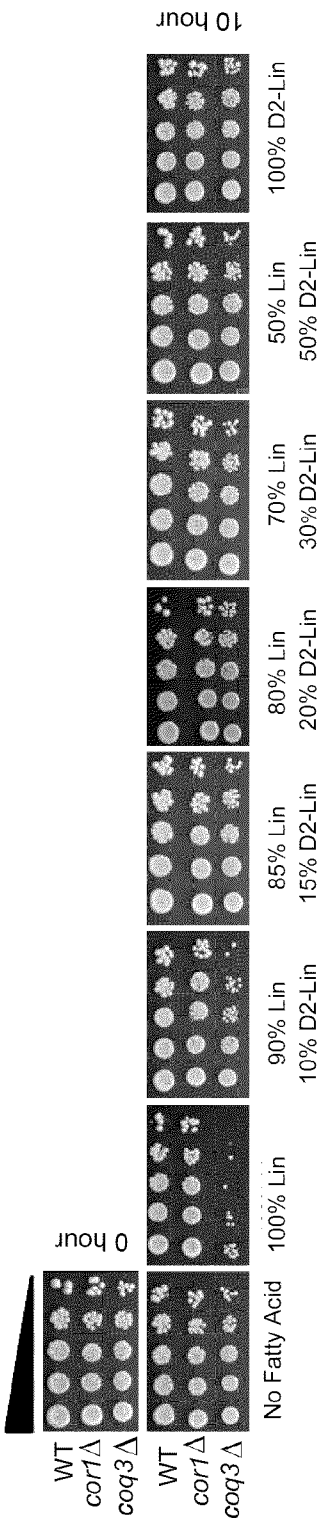

10 hour
0 hour
WT
cor1Δ
coq3 Δ
WT
cor1Δ
coq3 Δ
No Fatty Acid
100% Lin
90% Lin
10% D2-Lin
85% Lin
15% D2-Lin
80% Lin
20% D2-Lin
70% Lin
30% D2-Lin
50% Lin
50% D2-Lin
100% D2-Lin

FIG. 10

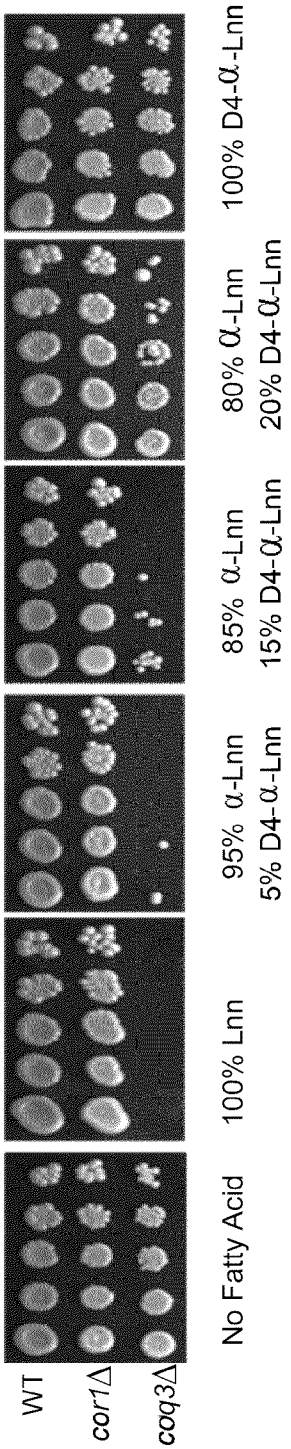
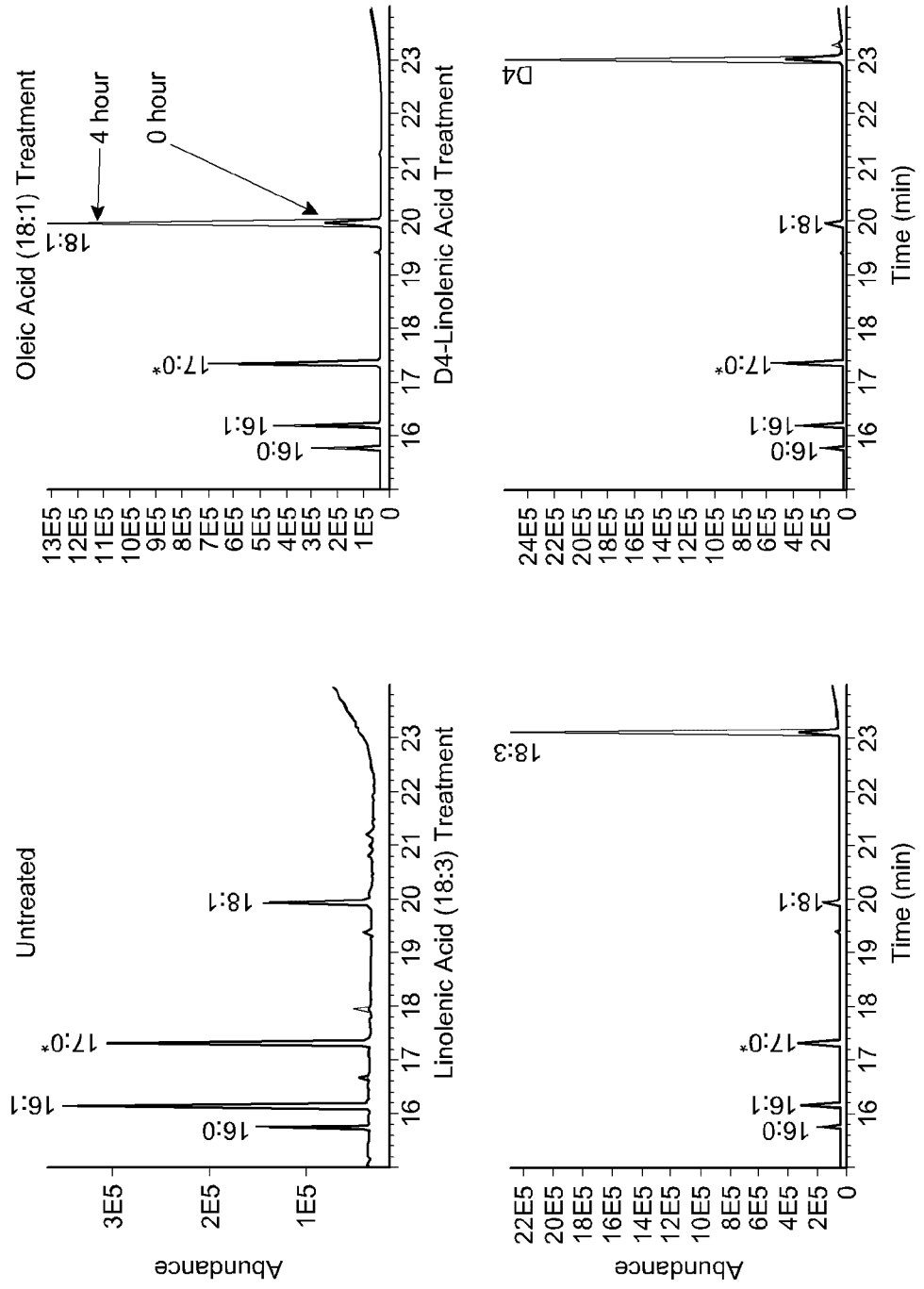

WT
cor1Δ
coq3Δ
No Fatty Acid
100% Lnn
95% α-Lnn
5% D4-α-Lnn
85% α-Lnn
15% D4-α-Lnn
80% α-Lnn
20% D4-α-Lnn
100% D4-α-Lnn

FIG. 11


Untreated
Linolenic Acid (18:3) Treatment
Oleic Acid (18:1) Treatment
D4-Linolenic Acid Treatment
4 hour
0 hour
D4
16:0
16:1
17:0*
18:1
18:3
Abundance
Time (min)

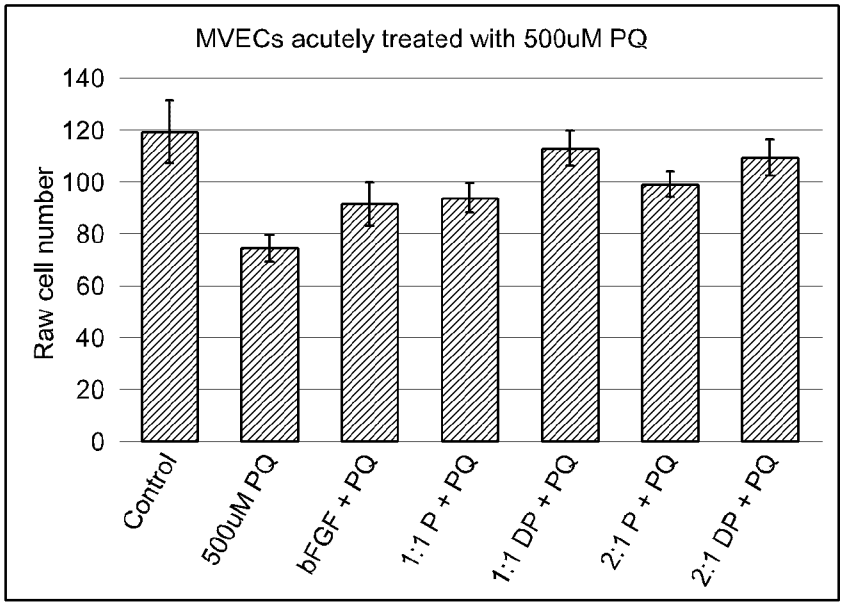

MVECs acutely treated with 500uM PQ
Raw cell number
140
120
100
80
60
40
20
0
Control
500uM PQ
bFGF + PQ
1:1 P + PQ
1:1 DP + PQ
2:1 P + PQ
2:1 DP + PQ

FIG. 12

FIG. 13

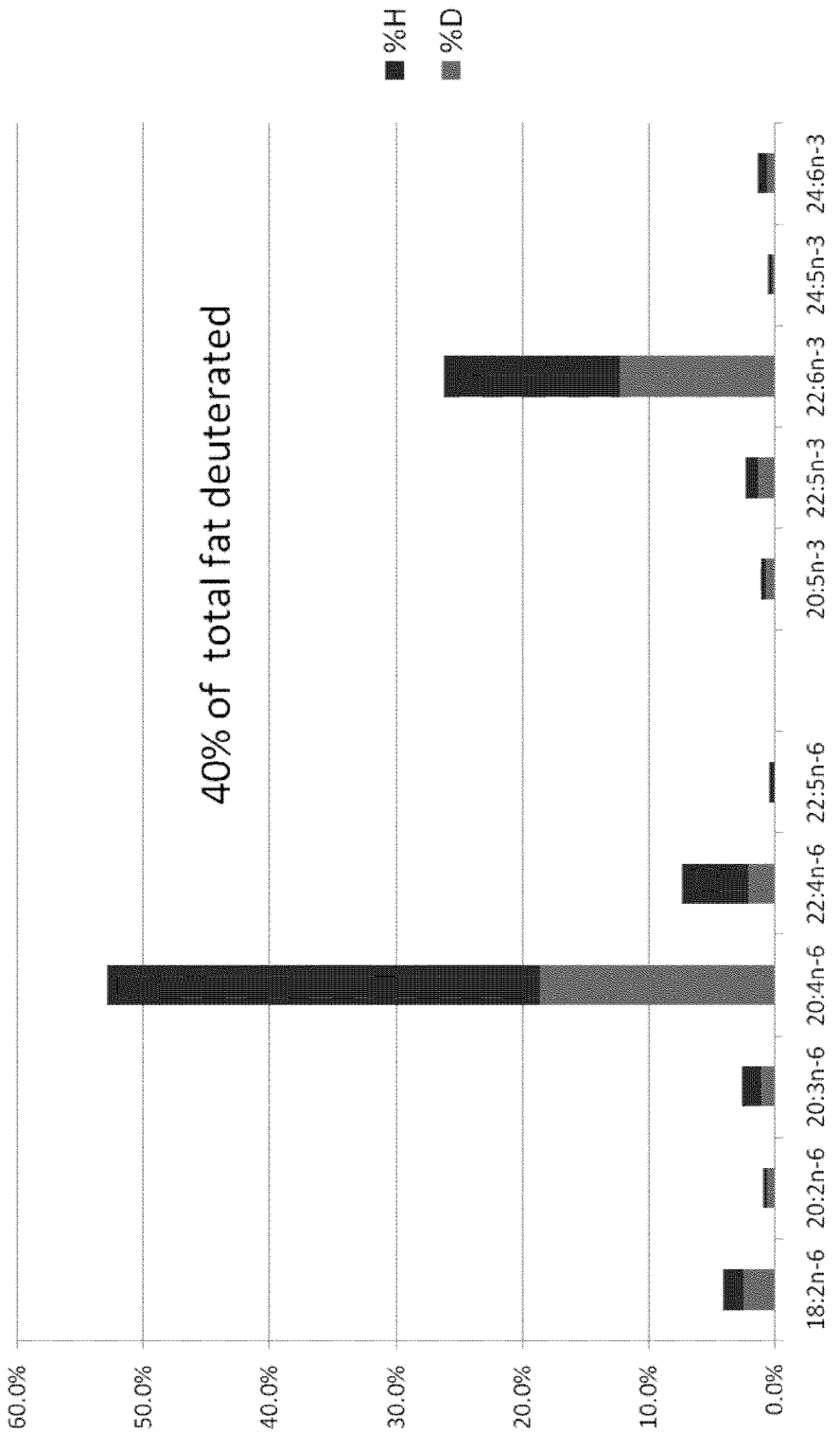

Retinal Fatty Acid Profile
Post 1:1 D-LA, D-ALA dosing
40% of total fat deuterated
%H
%D
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
18:2n-6
20:2n-6
20:3n-6
20:4n-6
22:4n-6
22:5n-6
20:5n-3
22:5n-3
22:6n-3
24:5n-3
24:6n-3

FIG. 14

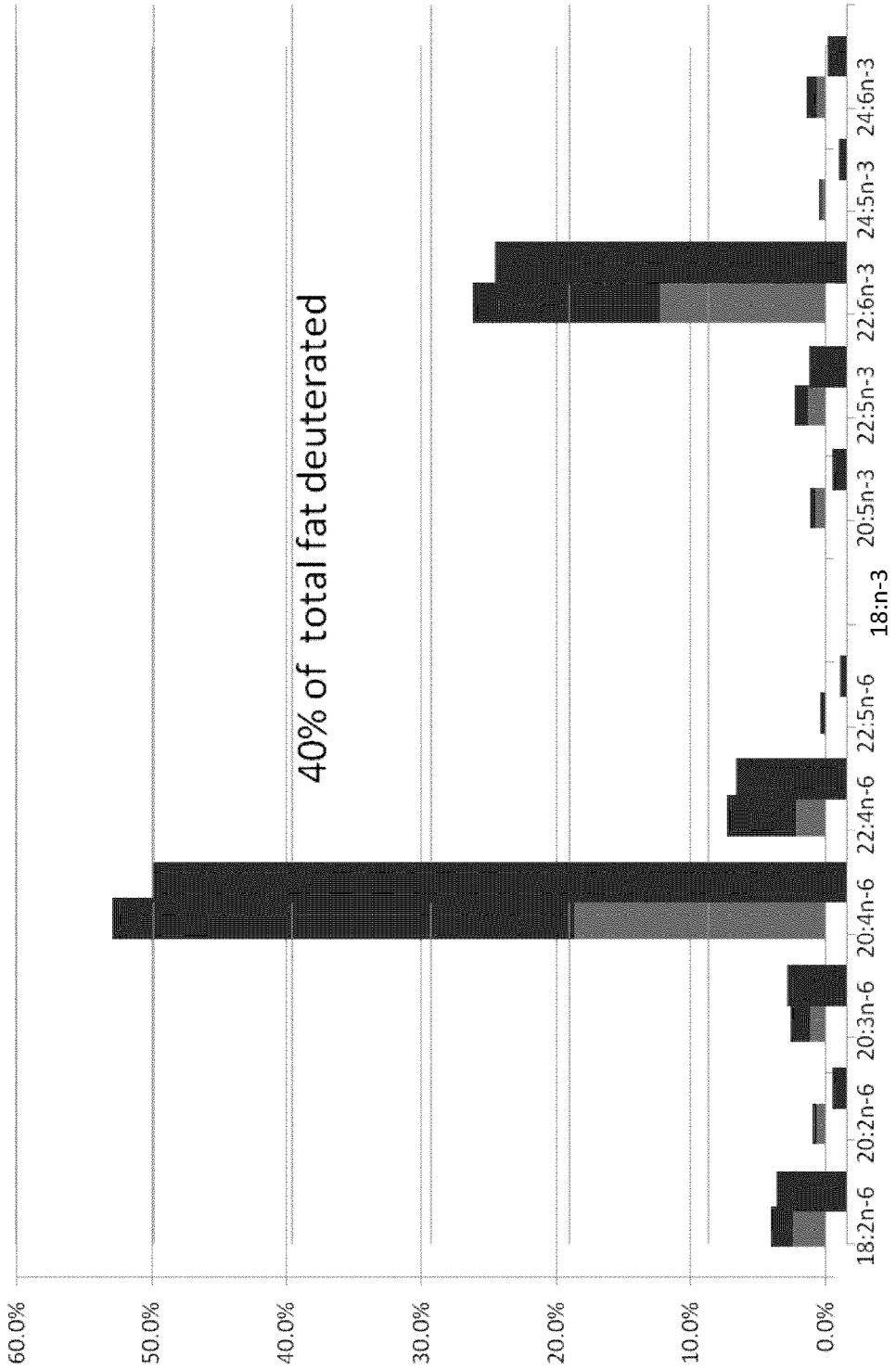

60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
40% of total fat deuterated
18:2n-6
20:2n-6
20:3n-6
20:4n-6
22:4n-6
22:5n-6
18:n-3
20:5n-3
22:5n-3
22:6n-3
24:5n-3
24:6n-3

FIG. 15

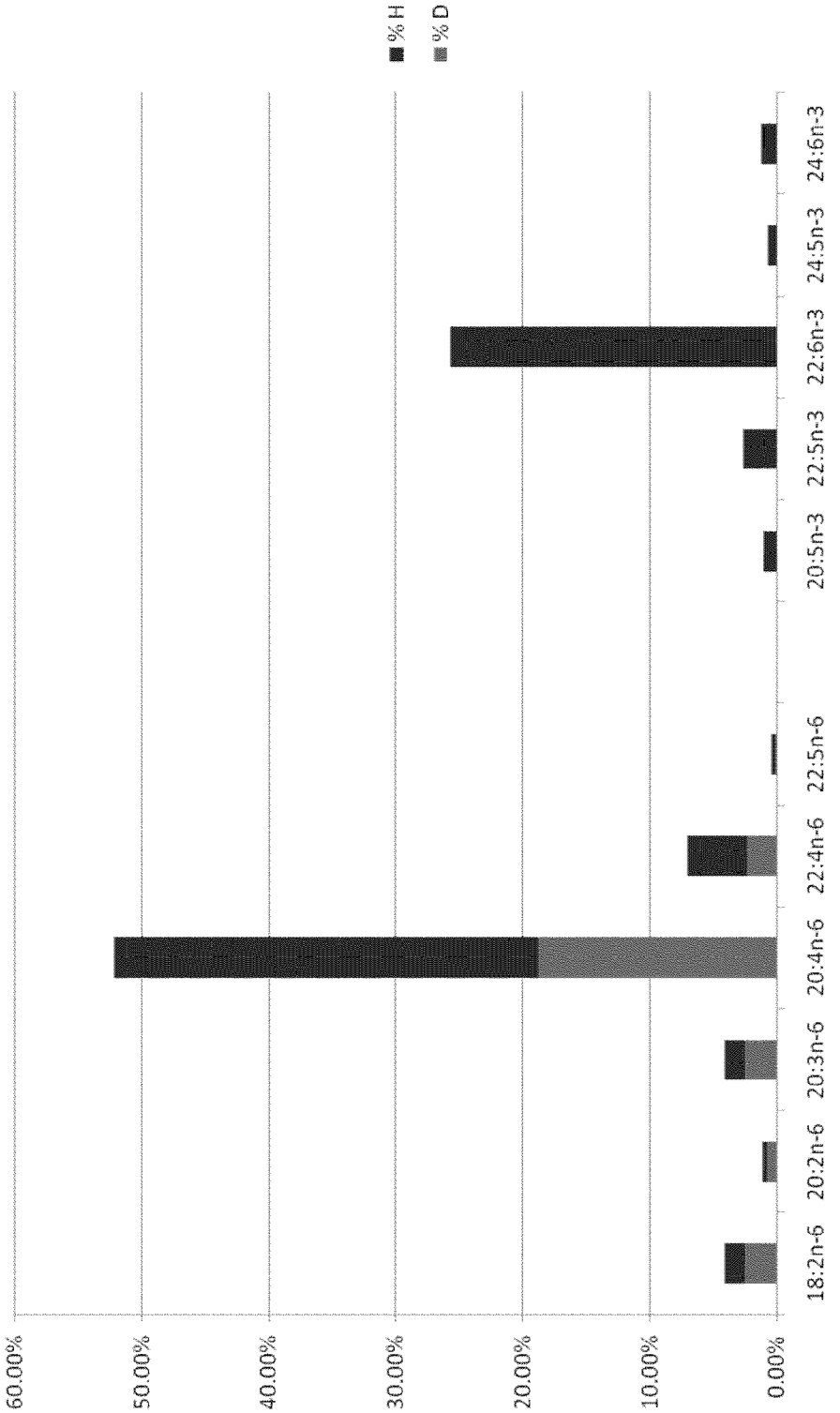

Retinal Fatty Acid Profile
Post Dosing 1:1 D-LA, H-ALA
% H
% D
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
18:2n-6
20:2n-6
20:3n-6
20:4n-6
22:4n-6
22:5n-6
20:5n-3
22:5n-3
22:6n-3
24:5n-3
24:6n-3

FIG. 16

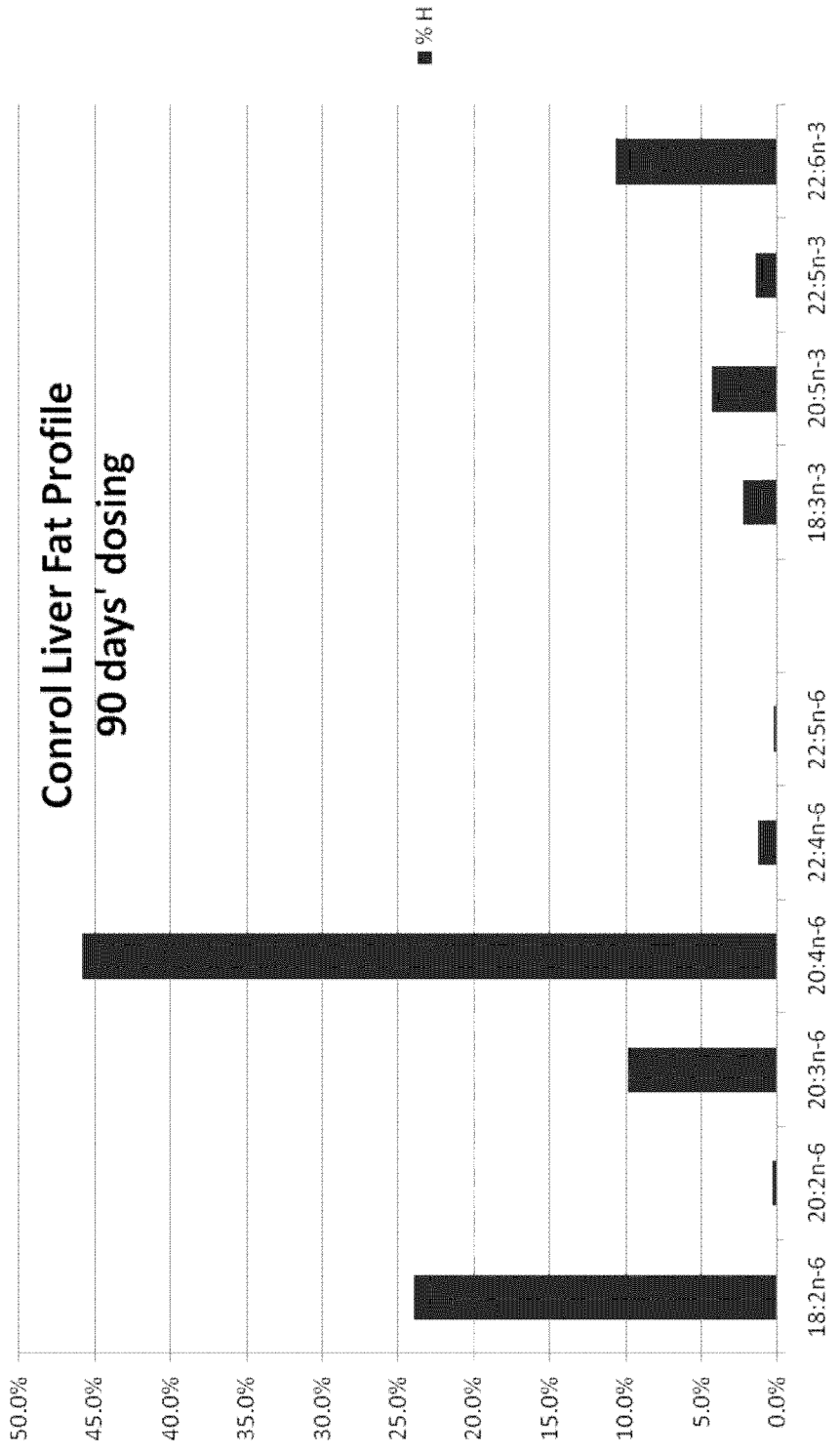

Conrol Liver Fat Profile
90 days' dosing
% H
50.0%
45.0%
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
18:2n-6
20:2n-6
20:3n-6
20:4n-6
22:4n-6
22:5n-6
18:3n-3
20:5n-3
22:5n-3
22:6n-3

FIG. 17

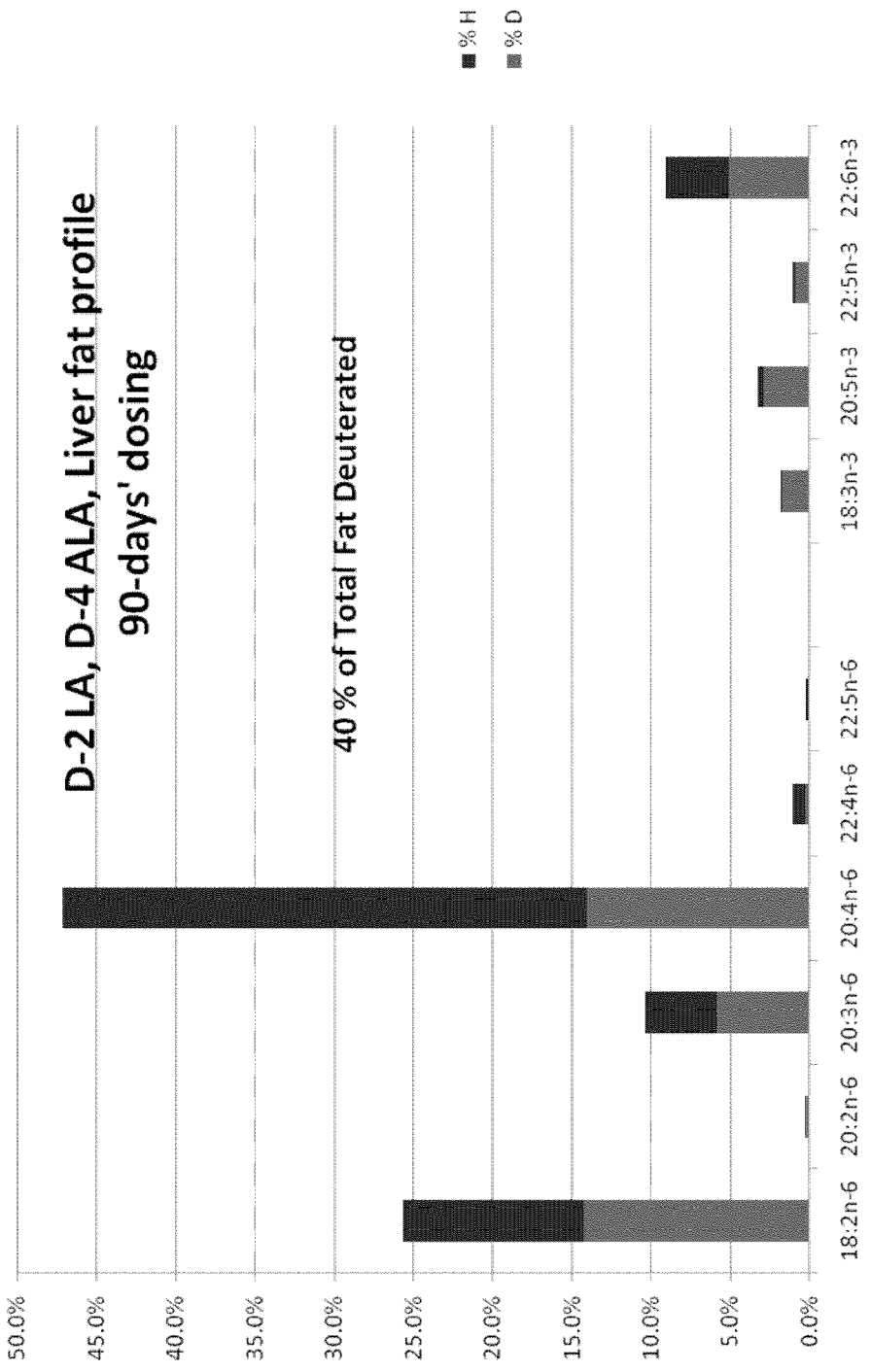

D-2 LA, D-4 ALA, Liver fat profile
90-days' dosing
40 % of Total Fat Deuterated
% H
% D
50.0%
45.0%
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
18:2n-6
20:2n-6
20:3n-6
20:4n-6
22:4n-6
22:5n-6
18:3n-3
20:5n-3
22:5n-3
22:6n-3

FIG. 18

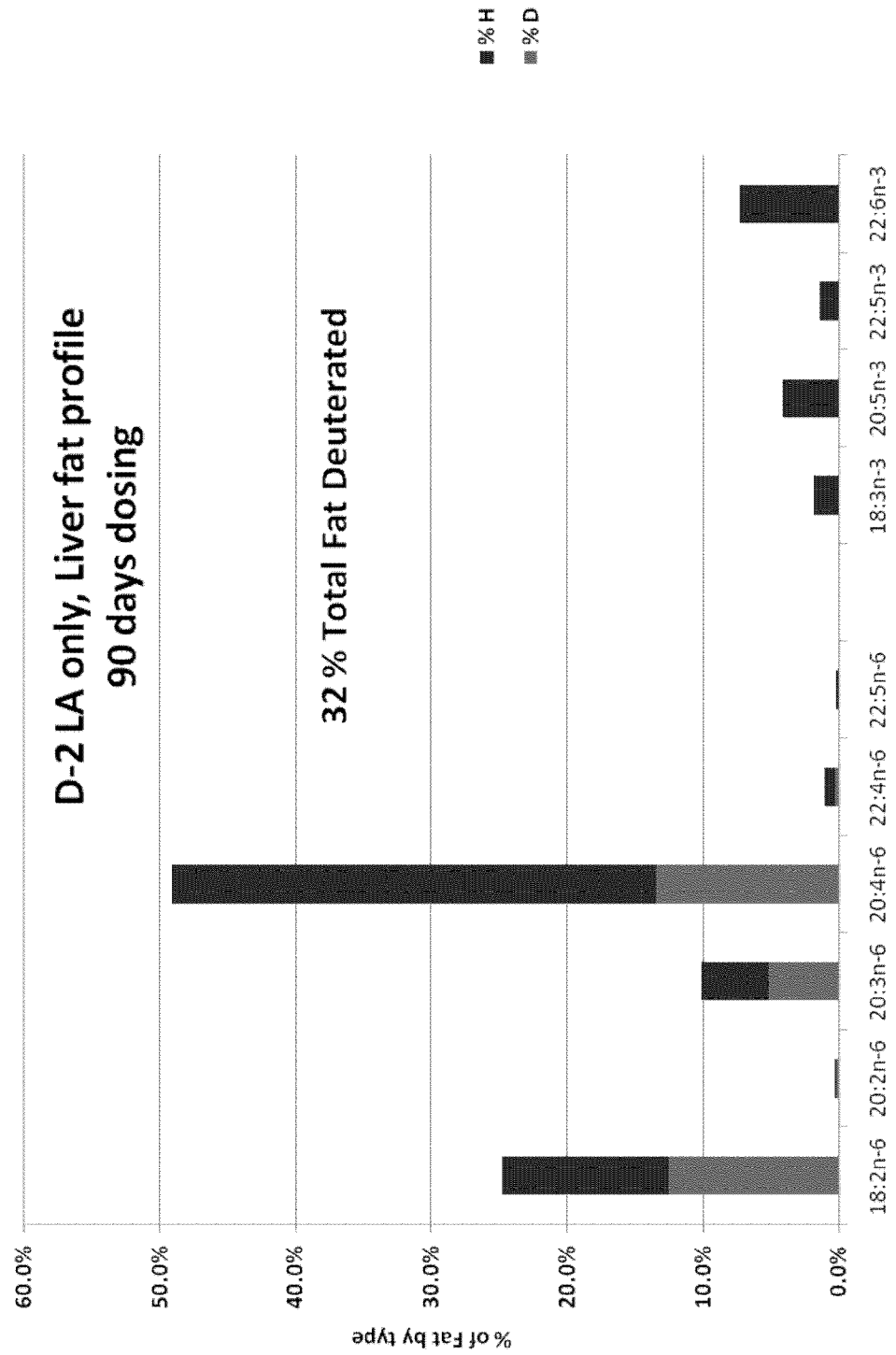

D-2 LA only, Liver fat profile
90 days dosing
32 % Total Fat Deuterated
% H
% D
% of Fat by type
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
18:2n-6
20:2n-6
20:3n-6
20:4n-6
22:4n-6
22:5n-6
18:3n-3
20:5n-3
22:5n-3
22:6n-3

FIG. 19

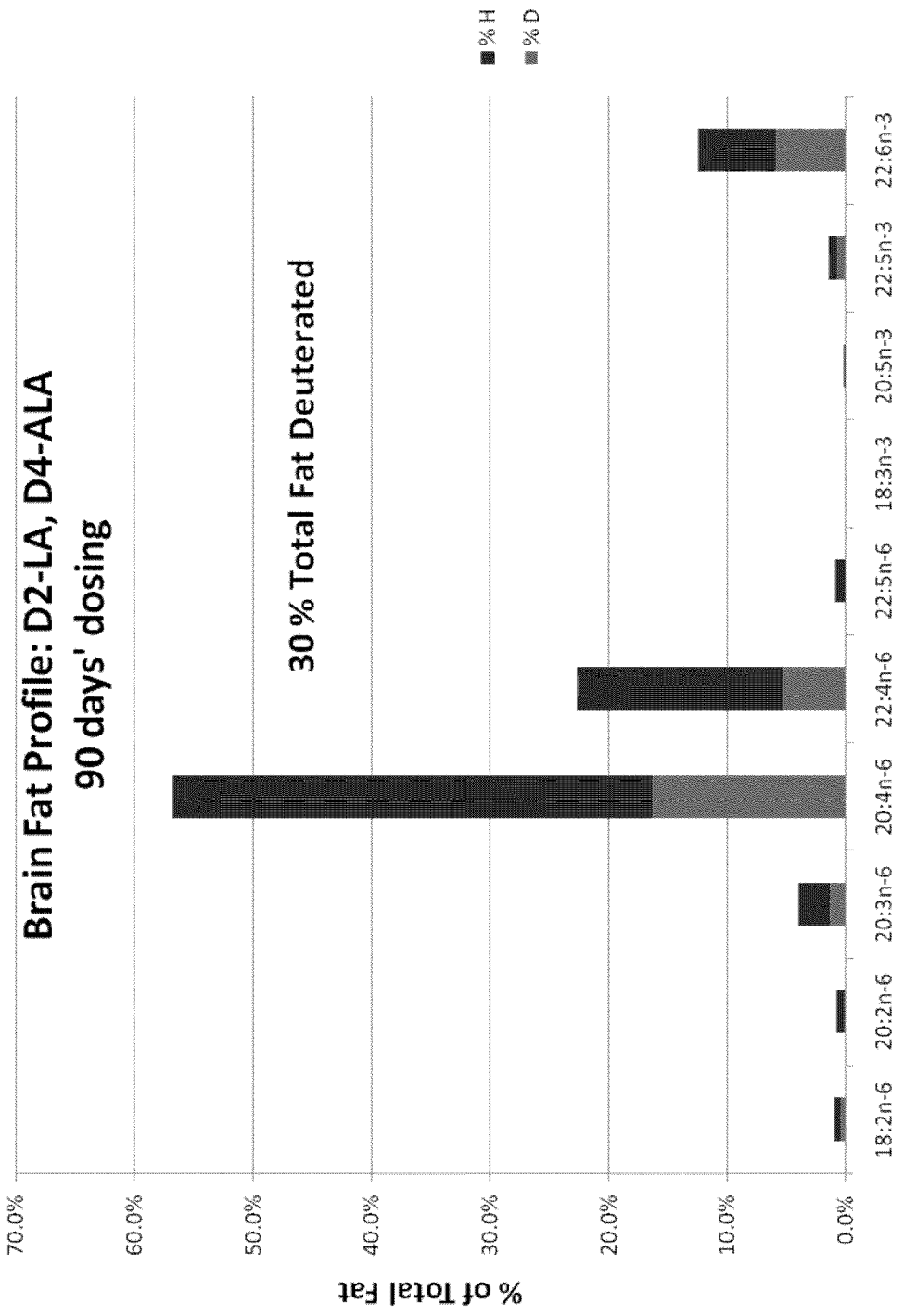

Brain Fat Profile: D2-LA, D4-ALA
90 days' dosing
30 % Total Fat Deuterated
% H
% D
% of Total Fat
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
18:2n-6
20:2n-6
20:3n-6
20:4n-6
22:4n-6
22:5n-6
18:3n-3
20:5n-3
22:5n-3
22:6n-3

FIG. 20

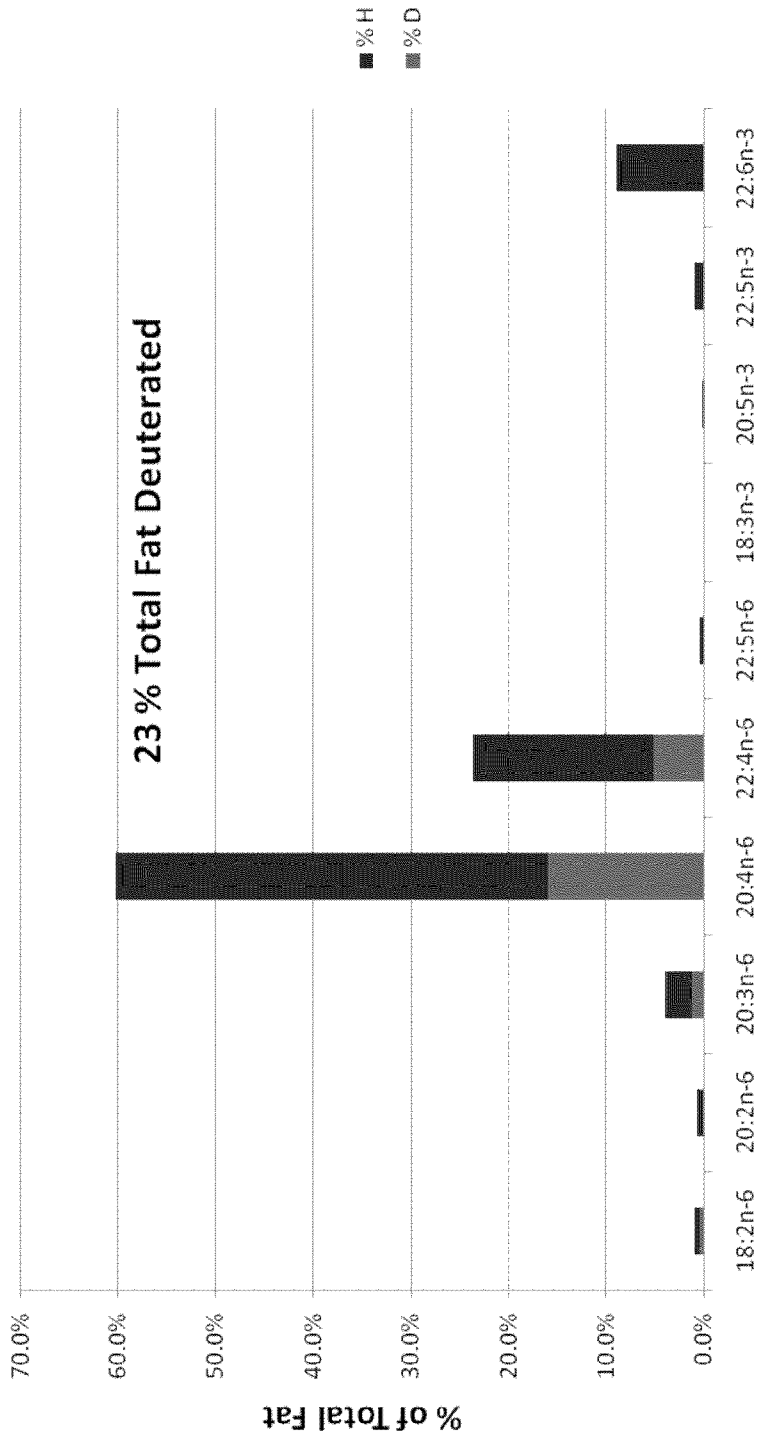
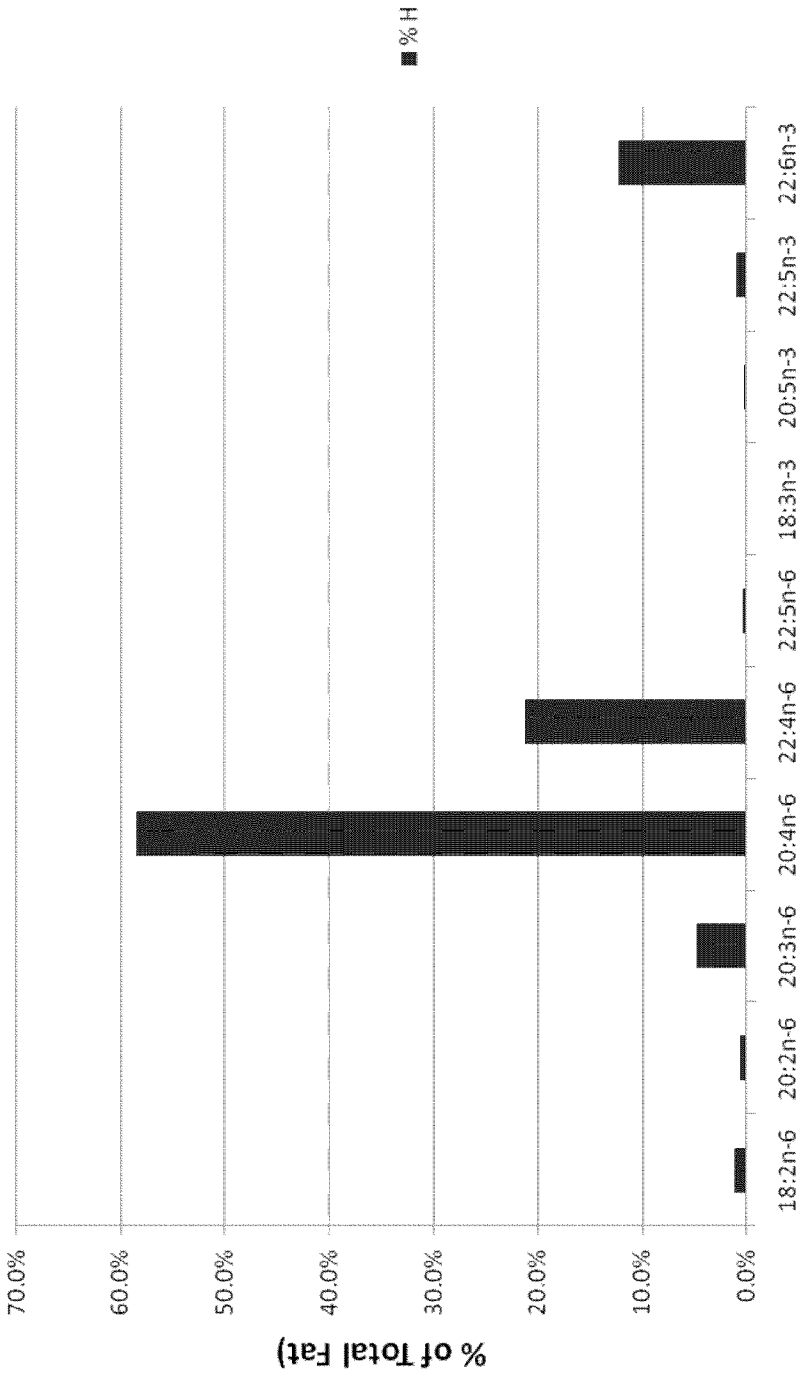
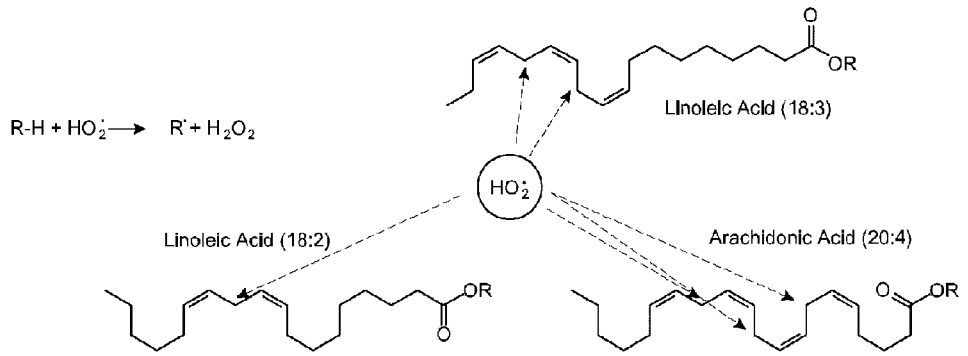

Brain Fat Profile: D2-LA, H4-ALA
90 days' dosing
23 % Total Fat Deuterated
% H
% D
% of Total Fat
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
18:2n-6
20:2n-6
20:3n-6
20:4n-6
22:4n-6
22:5n-6
18:3n-3
20:5n-3
22:5n-3
22:6n-3

FIG. 21


Brain Fat Profile: Control
90 days' dosing
% H
% of Total Fat)
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
18:2n-6
20:2n-6
20:3n-6
20:4n-6
22:4n-6
22:5n-6
18:3n-3
20:5n-3
22:5n-3
22:6n-3


R-H + HO2· ⟶ R· + H2O2
HO2·
Linolelc Acid (18:3)
Linoleic Acid (18:2)
Arachidonic Acid (20:4)
OR
O