The invention relates to compounds and methods for inhibiting production and function of 3-deoxyglucosone and other alpha-dicarbonyl sugars in skin, by way of fructoseamine-3-kinase inhibition, thereby treating or preventing various diseases, disorders or conditions. Additionally, the invention relates to treatment of various diseases, disorders or conditions associated with or mediated by oxidative stress since 3DG induces ROS and AGEs, which are associated with the inflammatory response caused by oxidative stress.
TITLE OF THE INVENTION
Compositions and Methods Related to Fructosamine-3-Kinase Inhibitors

BACKGROUND OF THE INVENTION

Biological amines react with reducing sugars to form a complex family of rearranged and dehydrated covalent adducts that include many cross-linked structures. Food chemists have long studied this process, referred to as glycation or the Maillard reaction, as a source of flavor, color, and texture changes in cooked, processed, and stored foods. However it is known that this process also occurs slowly in vivo. In a glycation reaction, alpha-dicarbonyl compounds such as deoxyglucosone, methylglyoxal, and glyoxal are more reactive than the parent sugars with respect to their ability to react with amino groups of proteins to form inter- and intramolecular cross-links of proteins, referred to as advanced glycation end products (AGEs or AGE-proteins). The formation of AGE-proteins from sugars is a multi-step process, involving early, reversible reactions with sugars to produce fructose-lysine containing proteins. These modified proteins then continue to react to produce irreversibly modified AGE-proteins. AGE-proteins are not identical to proteins containing glycated-lysine residues, as antibodies raised against AGE-proteins do not react with fructose-lysine.

The AGEs, which are irreversibly formed, accumulate with aging, atherosclerosis, and diabetes mellitus, and are especially associated with long-lived proteins such as collagens, lens crystallins, and nerve proteins. In the case of diabetic complications, the reactions that lead to AGE-proteins are thought to be kinetically accelerated by the chronic hyperglycemia associated with this disease. It has been shown that long-lived proteins such as collagen and lens crystallins from diabetic subjects contain a significantly greater AGE-protein content than do those from age-matched normal controls. Thus, the unusual incidence of cataracts in diabetics at a relatively early age, as well as the early onset of joint and arterial stiffening and loss of lung capacity observed in diabetics is explained by the increased rate of modification and cross-linking of these structural proteins. Likewise, diabetic retinopathy may be explained by the increased cross-linking of nerve proteins in the eye.
The alpha-dicarbonyl sugar 3-deoxyglucosone (3DG) is believed to be a key intermediate in the multistep pathway leading to formation of AGE-proteins. 3DG is a potent protein crosslinker and has been shown to be capable of inducing apoptosis, mutations, and formation of active oxygen species.

Many studies have concentrated on the role of 3DG in diabetes. It has been shown that diabetic humans have elevated levels of 3DG and 3-deoxyfructose (3DF), 3DG's detoxification product, in plasma (Niwa et al., 1993, Biochem. Biophys. Res. Commun. 196:837-843; Wells-Knecht et al., 1994, Diabetes. 43:1 152-1 156) and in urine (Wells-Knecht et al., 1994, Diabetes. 43:1152-1156), as compared with non-diabetic individuals. Furthermore, diabetics with nephropathy were found to have elevated plasma levels of 3DG compared to non-diabetics (Niwa et al., 1993, Biochem. Biophys. Res. Commun. 196:837-843). A recent study comparing patients with insulin-dependent diabetes mellitus (IDDM) and noninsulin-dependent diabetes mellitus (NIDDM) confirmed that 3DG and 3DF levels were elevated in blood and urine from both types of patient populations (Lal et al., 1995, Arch. Biochem. Biophys. 318:191-199). It has even been shown that incubation of glucose and proteins in vitro under physiological conditions produces 3DG. In turn, it has been demonstrated that 3DG glycates and crosslinks protein, creating detectable AGE products (Baynes et al., 1984, Methods Enzymol. 106:88-98; Dyer et al., 1991, J. Biol. Chem. 266:11654-11660). The normal pathway for reductive detoxification of 3DG (conversion to 3DF) may be impaired in diabetic humans since their ratio of urinary and plasma 3DG to 3DF differs significantly from non-diabetic individuals (Lal et al., 1995, Arch Biochem. Biophys. 318:191-199).

Furthermore, elevated levels of 3DG-modified proteins have been found in diabetic rat kidneys compared to control rat kidneys (Niwa et al., 1997, J. Clin. Invest. 99: 1272-1280). It has been demonstrated that 3DG has the ability to inactivate enzymes such as glutathione reductase, a central antioxidant enzyme. It has also been shown that hemoglobin-AGE levels are elevated in diabetic individuals (Makita et al., 1992, Science 258:651-653) and other AGE proteins have been shown in experimental models to accumulate with time, increasing from 5-50 fold over periods of 5-20 weeks in the retina, lens and renal cortex of diabetic rats (Brownlee et al., 1994, Diabetes 43:836-841). In addition, it has been demonstrated that 3DG is a teratogenic factor in diabetic embryopathy (Eriksson et al., 1998, Diabetes 47:1960-
One pathway for formation of 3DG comprises a reversible reaction between glucose and the ε-NH2 groups of lysine-containing proteins, forming a Schiff base (Brownlee et al., 1994, Diabetes 43:836-841). This Schiff base then rearranges to form a more stable ketoamine known as fructoselysine (FL3P) or the "Amadori product."

It was initially believed that 3DG production resulted exclusively from subsequent non-enzymatic rearrangement, dehydration, and fragmentation of the fructoselysine containing protein (Brownlee et al., 1994, Diabetes 43:836-841 and Makita et al., 1992, Science 258:651-653). But more recent work has shown that an enzymatic pathway for the production of 3DG also exists and that this pathway produces relatively high concentrations of 3DG in organs affected by diabetes (Brown et al., U.S. Pat. No. 6,004,958). In the enzymatic pathway, a specific kinase (referred to herein as fructoselysine kinase) converts fructose-lysine into fructose-lysine-3-phosphate (FL3P) in an ATP-dependent reaction, and the FL3P then breaks down to form free lysine, inorganic phosphate, and 3DG (Brown et al., U.S. Pat. No. 6,004,958). Methods have also been described for assessing diabetic risk, based on measuring components of the 3DG pathway (WO 99/64561).

U.S. Pat. No. 6,004,958 describes a class of compounds that inhibits the enzymatic conversion of fructose-lysine to FL3P, thereby inhibiting formation of 3DG and other alpha-dicarbonyl sugars produced via this pathway. Specific compounds that are representative of the class have also been described (Brown et al., WO 98/33492). For example, it was disclosed in WO 98/33492 that urinary or plasma 3DG can be reduced by meglumine, sorbitollysine, mannotolylsine, and galactitolylsine.

It was also disclosed in WO 98/33492 that diets high in glycated protein are harmful to the kidney and cause a decrease in birth rate. Additionally, the fructoselysine pathway was reported to be involved in kidney carcinogenesis (WO 98/33492) it was further suggested that diet and 3DG may play a role in carcinogenesis associated with the fructoselysine pathway (WO 00/24405; WO 00/62626).

Once formed, 3DG can be detoxified in the body by at least two pathways. In one pathway, 3DG is reduced to 3-deoxyfructose (3DF) by aldehyde reductase, and the 3DF is then efficiently excreted in urine (Takahashi et al., 1995, Biochemistry

Results of studies to date show that the efficiency of at least one of these enzymes, aldehyde reductase, is adversely affected in diabetes. When isolated from diabetic rat liver, this enzyme is glycated on lysine at positions 67, 84 and 140 and has a low catalytic efficiency when compared with the normal, unmodified enzyme (Takahashi et al., 1995, Biochemistry 34:1433). Since diabetic patients have higher ratios of glycated proteins than normoglycemic individuals they are likely to have both higher levels of 3DG and a reduced ability to detoxify this reactive molecule by reduction to 3DF. It has also been found that overexpression of aldehyde reductase protects PC12 cells from the cytotoxic effects of methylglyoxal or 3DG (Suzuki et al., 1998, J. Biochem. 123:353-357).

The mechanism by which aldehyde reductase works has been studied. These studies demonstrated that this important detoxification enzyme is inhibited by aldose reductase inhibitors (ARIs) (Barski et al., 1995, Biochemistry 34:11264). ARIs are currently under clinical investigation for their potential to reduce diabetic complications. These compounds, as a class, have shown some effect on short term diabetic complications. However, they lack clinical effect on long term diabetic complications and they worsen kidney function in rats fed a high protein diet. This finding is consistent with the newly discovered metabolic pathway for lysine recovery. For example, a high protein diet will increase the consumption of fructose-lysine, which in turn undergoes conversion into 3DG by the kidney lysine recovery pathway. The detoxification of the resulting 3DG by reduction to 3DF will be inhibited by ARIs therapy. Inhibiting 3DG detoxification will lead to increased 3DG levels, with a concomitant increase in kidney damage, as compared to rats not receiving ARs. This is because inhibition of the aldose reductase by the AR's would reduce availability of aldose reductase for reducing 3DG and 3DF.

Aminoguanidine, an agent that detoxifies 3DG pharmacologically via formation of rapidly excreted covalent derivatives (Hirsch et al., 1992, Carbohydr. Res. 232:125-130), has been shown to reduce AGE-associated retinal, neural, arterial, and renal pathologies in animal models (Brownlee et al., 1994, Diabetes 43:836-841; Brownlee et al., 1986, Science 232:1629-1632; Ellis et al., 1991, Metabolism
The role of alpha-dicarboxyl sugars and AGE-protein formation in diabetic complications has been extensively studied, as would be understood by the discussion presented above. But the pathogenic role of alpha-dicarboxyl sugars and AGE-proteins is not limited to diabetes. For example, protein glycation has been implicated in Alzheimer's disease (Harrington et al., Nature, 370: 247 (1994)). In addition, AGE-protein formation in vascular wall collagen appears to be an especially deleterious event, causing crosslinking of collagen molecules to each other and to circulating proteins. This leads to plaque formation, basement membrane thickening, and loss of vascular elasticity (Cerami & Ulrich, 2001, Recent Prog Horm Res:56:1-21). Increased protein fluorescence is also seen with aging. Some theories trace the aging process to a combination of oxidative damage and sugar-induced protein modification. Thus, a therapy that reduces AGE-protein formation may also be useful in treating other etiologically-similar human disease states, and perhaps slow the aging process.

In particular, Tobia and Kappler (U.S. Patent Publication No. 2003/0219440 Al) describe the effect of alpha-dicarboxyl sugars and AGE proteins on the condition and aging of skin. US 2003/0219440 reports that 3DG is present in human skin and that the gene encoding the enzyme regulating the synthesis of 3DG is expressed in skin. US 2003/0219440 discloses compositions and methods to inhibit enzymatically induced 3DG synthesis and accumulation in skin, as well as to inhibit 3DG function or increase the rate of detoxification and removal of 3DG from skin. Representative examples of those compositions and methods were purported to reduce collagen crosslinking in vitro and to improve skin elasticity in STZ diabetic rats.

A link between AGE-proteins and proinflammatory responses has also been established in diseases and disorders in which inflammation is a component. For example, AGEs contribute to kidney disease due to diabetes or aging by means of mesangial cell (MC) receptors, such as the receptor for AGE (RAGE), which promote oxidant-stress-dependent NF-κB activation and inflammatory gene expression (Lu et al., 2004, Proc Natl Acad Sci USA 32: 11767-11772). AGE cross-linking of proteins has been reported to contribute to the pathogenic cascade of cytokine- and intereferon-

It has been reported that a common form of AGE-proteins (N-ε(carboxymethyl) lysine (CML)-modified proteins) engage cellular AGE receptors (RAGE) in vitro and in vivo to activate key cell signaling pathways such as the transcription factor NF-κB, with subsequent modulation of gene expression (Kisslinger et al., 1999, J Biol Chem 274: 31740-31749). Those findings linked AGE-RAGE interaction to the development of accelerated vascular and inflammatory complications that typify disorders in which inflammation is an established component. It has also been reported that short exposure of mesothelial cells to even to a single glucose degradation product (e.g., 3DG) results in increased formation of AGEs, enhanced cytotoxic damage and a proinflammatory response, evidenced by increased VCAM-I expression and elevated production of IL-6 and IL-8 (Welten et al., 2003, Pent Dial Int. 23: 213-221).

As can be appreciated from the foregoing discussion, the detrimental conditions associated with AGE-proteins and their underlying causative agents, alpha-dicarbonyl sugars, in tissues are many and varied, and include inflammatory diseases and disorders. Though treatments for various inflammatory conditions are available, heretofore they have not been targeted to causative factors such as AGE-proteins and the compounds that lead to formation of AGE-proteins. Accordingly, a pressing need exists to identify and develop compositions and methods of treating inflammation that are directed to those underlying factors. Additionally, a need exists for the treatment of inflammation-related disorders, such as pain and itch, that are related to the metabolic pathways as described herein. The present invention meets these needs.

BRIEF SUMMARY OF THE INVENTION

The invention includes a method of inhibiting fructosamine-3-kinase (F3K) activity in the skin of a mammal, comprising administering to the mammal an effective amount of an inhibitor of F3K activity, wherein the inhibitor of F3K activity is not meglumine.

The invention also includes a method of preventing the formation of 3DG in a mammal comprising administering to a mammal an F3K inhibitor, wherein the F3K inhibitor is not meglumine.
The invention includes a n inhibitor administered via a route selected from the group consisting of topical, oral, rectal, vaginal, intramuscular, and intravenous.

The invention includes a method of inhibiting fructosamine-3-kinase activity, wherein the inhibitor comprises a compound of formula VIII:

\[
G^{10} - \text{VIII}
\]

wherein

\(G^{10}\) is independently selected at each occurrence from the group consisting of formulae \(\text{VIII}^1, \text{VIII}^2, \text{VIII}^3, \text{VIII}^4, \text{VIII}^5, \text{VIII}^6, \text{VIII}^7, \text{VIII}^8, \text{VIII}^9, \text{VIII}^{10}\):
R\textsuperscript{4} is independently selected at each occurrence from the group consisting of Hydrogen, -OH, -CH\textsubscript{2}OH, and -CH\textsubscript{3}.

The invention includes a method of inhibiting fructosamine-3-kinase activity, wherein the inhibitor is:

\[
\begin{align*}
\text{or a pharmaceutically acceptable salt thereof.}
\end{align*}
\]

The invention includes a method of inhibiting fructosamine-3-kinase activity, wherein the inhibitor comprises a compound of formula X:

\[
\text{X}
\]

wherein

R\textsuperscript{5} is independently selected at each occurrence from the group consisting of Hydrogen; F; Cl; Br; I; (Ci-C\textsubscript{6})alkyl; (Ci-C\textsubscript{6})alkenyl; (Ci-C\textsubscript{6})alkoxy; OH; NO\textsubscript{2}; C=N; C(=O)(Ci-C\textsubscript{3})alkyl; (C\textsubscript{2}-C\textsubscript{6})alkylene-OR\textsubscript{2}; phosphonato; NR\textsubscript{2}; NH(=O)(Ci-C\textsubscript{3})alkyl; sulfamyl; carbamyl; OC(=O)(Ci-C\textsubscript{3})alkyl; O(C\textsubscript{2}-C\textsubscript{6})alkylene-N((Ci-C\textsubscript{6})alkyl)\textsubscript{2}; and (Ci-C\textsubscript{3})perfluoroalkyl; 0((C\textsubscript{0}-C\textsubscript{6})Alkyl)Ar;

R\textsuperscript{2} independently selected at each occurrence from the group consisting of hydrogen and (Ci-C\textsubscript{6})alkyl;

Ar is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; (Ci-C\textsubscript{6})alkyl; (Ci-C\textsubscript{6})alkenyl; (Ci-C\textsubscript{6})alkoxy; OH; NO\textsubscript{2}; C=N; C(=O)(Ci-C\textsubscript{3})alkyl; (C\textsubscript{2}-C\textsubscript{6})alkylene-OR\textsubscript{2}; phosphonato; NR\textsubscript{2}; NH(=O)(Ci-C\textsubscript{3})alkyl; sulfamyl; carbamyl; OC(=O)(Ci-C\textsubscript{3})alkyl; O(C\textsubscript{2}-C\textsubscript{6})alkylene-N((Ci-C\textsubscript{6})alkyl)\textsubscript{2}; and (Ci-C\textsubscript{3})perfluoroalkyl;
or a stereoisomer or pharmaceutically acceptable salt of such a compound.

The invention includes a method of inhibiting fructosamine-3-kinase activity, wherein the inhibitor is:

![Chemical structure](image)

or a pharmaceutically acceptable salt thereof.

The invention includes a method of inhibiting fructosamine-3-kinase activity, wherein the inhibitor comprises a compound of formula IX:

![Chemical structure](image)

wherein

$R^5$ is independently selected at each occurrence from the group consisting of Hydrogen; F; Cl; Br; I; (C$_1$-C$_6$)alkyl; (C$_1$-C$_3$)alkenyl; (C$_1$-C$_6$)alkoxy; OH; NO$_2$; C≡N; C(=O)(C$_1$-C$_3$)alkyl; (C$_2$-C$_6$)alkylene-OR$^2$; phosphonato; NR$_2$; NHC=O(C$_1$-C$_3$)alkyl; sulfamyl; carbamyl; 0C(=O)(C$_1$-C$_3$)alkyl; O(C$_2$-C$_6$)alkylene-N((C$_1$-C$_6$)alkyl)$_2$; and (C$_1$-C$_3$)perfluoroalkyl; 0((C$_1$-C$_6$)Alkyl)Ar;

$R^2$ independently selected at each occurrence from the group consisting of hydrogen and (C$_1$-C$_6$)alkyl;

$A$r is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; (C$_1$-C$_6$)alkyl; (C$_1$-C$_6$)alkenyl; (C$_1$-C$_6$)alkoxy; OH; NO$_2$; C≡N; C(=O)(C$_1$-C$_3$)alkyl; (C$_2$-C$_6$)alkylene-OR$^2$; phosphonato; NR$_2$; NHC(=O)(C$_1$-C$_6$)alkyl; sulfamyl; carbamyl; OC(=O)(C$_1$-C$_3$)alkyl; O(C$_2$-C$_6$)alkylene-N((C$_1$-C$_6$)alkyl)$_2$; and (C$_1$-C$_3$)perfluoroalkyl;

or a stereoisomer or pharmaceutically acceptable salt of such a compound.
In an aspect of the invention, a mammal to which the compound is administered is a human.

In an aspect of the invention, the inhibitor comprises from about 0.0001% to about 15% by weight of a pharmaceutical composition.

In an aspect of the invention, an inhibitor is administered as a controlled-release formulation.

In an aspect of the invention, a pharmaceutical composition is selected from the group consisting of a lotion, a cream, a gel, a liniment, an ointment, a paste, a solution, a powder, and a suspension. A composition may further comprise a moisturizer, a humectant, a demulcent, oil, water, an emulsifier, a thickener, a thinner, a surface active agent, a fragrance, a preservative, an antioxidant, a hydrotropic agent, a chelating agent, a vitamin, a mineral, a permeation enhancer, a cosmetic adjuvant, a bleaching agent, a depigmentation agent, a foaming agent, a conditioner, a viscosifier, a buffering agent, and a sunscreen.

In an aspect of the invention, a compound inhibits advanced glycation end product modified protein formation. In another aspect, a compound inhibits a function selected from the group consisting of protein crosslinking, apoptosis, formation of reactive oxygen species, and mutagenesis. In yet another aspect, a compound stimulates 3DG detoxification. In still another aspect, a compound stimulates 3DG clearance.

The invention includes a method of treating an alpha-dicarbonyl sugar associated skin disease or disorder in a mammal comprising, administering to a mammal an alpha-dicarbonyl sugar inhibiting amount of a compound which inhibits F3K activity, thereby treating an alpha-dicarbonyl sugar associated skin disease or disorder of a mammal, wherein the inhibitor of F3K activity is not meglumine. In an aspect of the invention, an alpha-dicarbonyl sugar associated skin disease or disorder comprises a disease or disorder associated with a function selected from the group consisting of protein crosslinking, apoptosis, mutagenesis, and formation of reactive oxygen species. In another aspect, an alpha-dicarbonyl sugar associated skin disease or disorder comprises a disease or disorder associated with advanced glycation end product modified protein formation. In still another aspect, a disease or disorder is
selected from the group consisting of skin cancer, psoriasis, skin aging, skin wrinkling, hyperkeratosis, hyperplasia, acanthosis, papillomatosis, dermatosis, rhinophyma, scleroderma, eczema, seborrhea, and rosacea.

In an aspect of the invention, a compound is administered in combination with a topical steroid. A topical steroid of the invention includes hydrocortisone, clobetasone butyrate, triamcinolone acetonide, fluocinolone acetonide, betamethasone valerate, betamethasone dipropionate, diflucortolone valerate, fluticasone valerate, hydrocortisone 17-butyrate, mometasone furoate, methylprednisolone aceponate, betamethasone dipropionate, and clobetasol propionate, among others.

In an aspect of the invention, an alpha-dicarbonyl sugar associated skin disease or disorder comprises a disease or disorder associated with acne. In an aspect, a compound is administered in combination with at least one additional composition for treating acne. Such compositions include, but are not limited to, benzoyl peroxide, salicylic acid and erythromycin.

In an aspect of the invention, a composition further comprises at least one of the members selected from the group consisting of an antacid, a probiotic agent, an H-2 blockers, and a proton pump inhibitor.

In an aspect, a composition further comprises arginine.

In an aspect of the invention, a composition further comprises a non-steroidal anti inflammatory drug (NSAID). In an aspect, a non-steroidal anti inflammatory drug (NSAID) is selected from the group consisting of ibuprofen (2-(isobutylphenyl)-propionic acid); methotrexate (N-[4-(2,4 diamino 6-pteridinyl-methyl]methylamino]benzoyl)-L-glutamic acid); aspirin (acetylsalicylic acid); salicylic acid; diphenhydramine (2-(diphenylmethoxy)-NN-dimethylethylamine hydrochloride); naproxen (2-naphthaleneacetic acid, 6-methoxy-9-methyl-, sodium salt, (-)); phenylbutazone (4-butyl-1,2-diphenyl-3,5- pyrazolidineone); sulindac-(2)-5-fluo-ro-2-methyl-L-[[p-(methylsulfinyl)phenyl]methylene-]-IH-indene-3-acetic acid; diflunisal (2',4',-difluoro-4-hydroxy-3-biphenylcarboxylic acid; piroxicam (4-hydroxy-2-methyl-N-2-pyridinyl-2H-1 ,2-benzothiazine-2-carboxamide 1,1-dioxide, an oxicam; indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-H-indole-3-acetic acid); meclofenamate sodium (N-(2,6-dichloro-m-tolyl) anthranilic acid, sodium salt, monohydrate); ketoprofen (2-(3-benzoylphenyl)-propionic acid; tolmetin
sodium (sodium l-methyl-5-(4-methylbenzoyl-lH-pyrrole-2-acetate dihydrate);
diclofenac sodium (2-[(2,6-dichlorophenyl)amino]benzeneacetic acid, monosodium salt); hydroxychloroquine sulphate (2-{[4-[(7-chloro-4-quinolyl)amino]pentyl]ethylamino}ethanol sulfate (1:1); penicillamine (3-mercaptop-D-valine);
flurbiprofen ([l,l-biphenyl]-4-acetic acid, 2-fluoro-alpha-methyl- (+-)); cetodolac (1-8-diethyl-13,4,9, tetrahydropyrano-[3-4-13]indole-1-acetic acid; mefenamic acid (N-(2,3-xylyl)anthranilic acid; and diphenhydramine hydrochloride (2-diphenylmethoxy-N,N-di-methylethamine hydrochloride).

The invention includes kit for administering a compound which inhibits F3K activity in the skin of a mammal comprising a compound which inhibits F3K activity, a standard, an applicator, and an instructional material for the use thereof, wherein the inhibitor of F3K activity is not meglumine. In an aspect, the mammal is a human.

The invention includes a method of treating a disease associated with the presence of 3DG in a mammal comprising administering to a mammal a composition comprising an F3K inhibitor, wherein the F3K inhibitor is not meglumine.

The invention includes a method of treating an inflammatory condition in a mammal comprising administering to the mammal a composition comprising an F3K inhibitor, the administration resulting in reduction or elimination of the alpha-dicarbonyl sugar at a site in the mammal, wherein the site is affected by the inflammatory condition, thereby treating the inflammatory condition, wherein the F3K inhibitor is not meglumine. In an aspect, the inflammatory condition is selected from the group consisting of allergic conditions, Alzheimer's disease, anemia, angiogenesis, aortic valve stenosis, atherosclerosis, thrombosis, rheumatoid arthritis, osteoarthritis, gout, gouty arthritis, acute pseudogout, acute gouty arthritis, inflammation associated with cancer, congestive heart failure, cystitis, fibromyalgia, fibrosis, glomerulonephritis, inflammation associated with gastro-intestinal disease, inflammatory bowel diseases, irritable bowel diseases, kidney failure, glomerulonephritis, myocardial infarction, ocular diseases, pancreatitis, psoriasis, reperfusion injury or damage, respiratory disorders, restenosis, septic shock, endotoxic shock, urosepsis, stroke, surgical complications, systemic lupus.
The invention includes a method of treating pain in a mammal comprising administering to the mammal a composition comprising an F3K inhibitor, the administration resulting in reduction or elimination of the alpha-dicarbonyl sugar at a site in the mammal, wherein the site is affected by the pain, thereby treating the pain, wherein the F3K inhibitor is not meglumine. In an aspect, the pain is selected from the group consisting of arachnoiditis, arthritis, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, gout, tendonitis, bursitis, sciatica, spondylolisthesis, radiculopathy, burn pain, cancer pain, headaches, migraines, cluster headaches, tension headaches, trigeminal neuralgia, myofascial pain, neuropathic pain, pain associated with diabetic neuropathy, reflex sympathetic dystrophy syndrome, phantom limb pain, post-amputation pain, tendonitis, tenosynovitis, postherpetic neuralgia, shingles-associated pain, central pain syndrome, trauma-associated pain, vasculitis, pain associated with infections, skin tumors, cysts, pain associated with tumors associated with neurofibromatosis, pain associated with strains, bruises, dislocations, fractures, and pain due to exposure to chemicals.

In another aspect, the cancer is selected from the group consisting of NSCLC, ovarian cancer, pancreatic cancer, breast carcinoma, colon carcinoma, rectum carcinoma, lung carcinoma, oropharynx carcinoma, hypopharynx carcinoma, esophagus carcinoma, stomach carcinoma, pancreas carcinoma, liver carcinoma, gallbladder carcinoma, bile duct carcinoma, small intestine carcinoma, urinary tract carcinoma, kidney carcinoma, bladder carcinoma, urothelial carcinoma, female genital tract carcinoma, cervix carcinoma, uterus carcinoma, ovarian carcinoma, choriocarcinoma, gestational trophoblastic disease, male genital tract carcinoma, prostate carcinoma, seminal vesicles carcinoma, testes carcinoma, germ cell tumors, endocrine gland carcinoma, thyroid carcinoma, adrenal carcinoma, pituitary gland carcinoma, skin carcinoma, hemangiomas, melanomas, sarcomas, bone and soft tissue sarcoma, Kaposi's sarcoma, tumors of the brain, tumors of the nerves, tumors of the eyes, tumors of the meninges, astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, meningiomas, solid tumors arising from hematopoietic malignancies, and solid tumors arising from lymphomas.
The invention includes a method of treating itch in a mammal comprising administering to the mammal a composition comprising an F3K inhibitor, the administration resulting in reduction or elimination of the alpha-dicarbonyl sugar at a site in the mammal, wherein the site is affected by the itch, thereby treating the itch, wherein the F3K inhibitor is not meglumine. In an aspect, the itch is the result of a condition selected from the group consisting of cutaneous itch, neuropathic itch, neurogenic itch, mixed-type itch, and psychogenic itch.

In an aspect of the invention, solid tumors arising from hematopoietic malignancies is selected from the group consisting of leukemias, chloromas, plasmacytomas and the plaques and tumors of mycosis fungoides and cutaneous T-cell lymphoma/leukemia.

In an aspect of the invention, a gastro-intestinal disease is selected from the group consisting of aphthous ulcers, pharyngitis, esophagitis, peptic ulcers, gingivitis, periodontitis, oral mucositis, gastrointestinal mucositis, nasal mucositis, irritable bowel disease and proctitis.

In an aspect of the invention, an inflammatory bowel disease is selected from the group consisting of Crohn's disease, ulcerative colitis, indeterminate colitis, necrotizing enterocolitis, pouchitis and infectious colitis.

In an aspect of the invention, an ocular disease is selected from the group consisting of conjunctivitis, retinitis, and uveitis.

In an aspect of the invention, a respiratory disorder is selected from the group consisting of asthma, mononuclear-phagocyte dependent lung injury, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, adult respiratory distress syndrome, acute chest syndrome in sickle cell disease, cystic fibrosis.

**BRIEF DESCRIPTION OF THE DRAWINGS**

The foregoing summary, as well as the following detailed description of preferred embodiments of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown. In the drawings:
Figure 1 is a schematic diagram depicting the initial step involved in the multi-step reaction leading to crosslinking of proteins.

Figure 2 is a schematic diagram which illustrates the reactions involved in the lysine recovery pathway. Fructose-lysine (FL) is phosphorylated by a fructosamine kinase such as amadorase to form fructoselysine 3-phosphate (FL3P). FL3P spontaneously decomposes into lysine, Pi, and 3DG (Brown et al., U.S. Patent No. 6,004,958).

Figure 3 is a graph representing a urinary profile showing the variation over time of 3DF, 3DG and FL from a single individual fed 2 grams of FL and followed for 24 hours.

Figure 4 is a graph representing 3DF excretion in urine over time from seven volunteers fed 2 grams of fructoselysine.

Figure 5 graphically compares 3DF and N-acetyl-β-glucosaminidase (NAG) levels in control animals and an experimental group maintained on feed containing 0.3% glycated protein (Brown et al.).

Figure 6 is a graph which demonstrates the linear relationship between 3DF and 3DG levels in urine of rats fed either a control diet or a diet enriched in glycated protein (Brown et al., U.S. Patent No. 6,004,958).

Figure 7, comprising Figure 7A and Figure 7B, graphically depicts fasting levels of urinary 3DG in normal subjects and in diabetic patients, plotted against the fasting level of 3DF.

Figure 8, comprising Figure 8A and Figure 8B, depicts images of photomicrographs illustrating the effects of a diet containing high levels of glycated protein on the kidney. Periodic acid and Schiff (PAS) stained kidney sections were prepared from a rat fed a diet enriched in mildly glycated protein (Figure 8A) and a rat fed a normal diet (Figure 8B). In this experiment, non-diabetic rats were fed a diet containing 3% glycated protein for 8 months. This diet substantially elevated levels of FL and its metabolites (>3-fold in the kidney). Figure 8A is an image of a photomicrograph of a glomerulus from a rat fed the glycated diet for 8 months. The glomerulus shows segmental sclerosis of the glomerular tuft with adhesion of the sclerotic area to Bowman's capsule (lower left). There is also tubular metaplasia of the parietal epithelia from approximately 9 to 3 o'clock. These sclerotic and metaplastic changes are reminiscent of the pathologies observed in diabetic kidney
disease. Figure 8B is an image from a rat on the control diet for 8 months, comprising a histologically normal glomerulus.

Figure 9 is a graphic comparison of 3DG and 3DF levels in glomerular and tubular fractions from rat kidneys after FL feeding.

Figure 10 is an image depicting the nucleic acid sequence (SEQ ID NO:1) of human amadorase (fructosamine-3-kinase), NCBI accession number NM_022158. The accession number for the human gene on chromosome 17 is NTJ) 10663.

Figure 11 is an image depicting the amino acid sequence (SEQ ID NO:2) of human amadorase (fructosamine-3-kinase), NCBI accession number NP_071441.

Figure 12 is an image of a polyacrylamide gel demonstrating the effects of 3DG on collagen crosslinking and the inhibition of 3DG induced crosslinking by arginine. Collagen type I was treated with 3DG in the presence or absence of arginine. The samples were subjected to cyanogen bromide (CNBr) digestion, electrophoresed on a 16.5% SDS Tris-tricine gel, and then the gels were processed using silver stain techniques to visualize the proteins. Lane 1 contains molecular weight marker standards. Lanes 2 and 5 contain 10 and 20 µl of the collagen mixture following CNBr digestion. Lanes 3 and 6 contain the collagen mixture treated with 3DG and then digested with CNBr, and loaded at 10 and 20 µl, respectively. Lanes 4 and 7 contain the mixture of collagen incubated with 5 mM 3DG and 10 mM arginine and then digested with CNBr, and loaded at 10 and 20 µl, respectively.

Figure 13 is an image of an agarose gel demonstrating that the mRNA for amadorase/fructosamine kinase is present in human skin. RT-PCR was utilized and published amadorase sequences were used as the basis for preparing templates for PCR. Based on the primers used (see Examples) for the PCR reaction, the presence of a 519 bp fragment in the gel indicates the presence of amadorase mRNA. Expression of amadorase, as based on the presence of amadorase mRNA indicated by a 519 bp fragment, was found in the kidney (lane 1) and in the skin (lane 3). No 519 bp fragments were found in the control lanes, which contained primer but no template (lanes 2 and 4). Lane 5 contained DNA molecular weight markers.
Figure 14 is a graphic illustration of the effects of DYN 12 (3-O-methylsorbitollysine) treatment on skin elasticity. Diabetic or normal rats were treated with DYN 12 (50 mg/kg daily) or saline for eight weeks and then subjected to skin elasticity tests. The four groups used included diabetic controls (saline injection; solid black bar), diabetics treated with DYN 12 (open bar), normal animal controls (saline injections; stippled bar), and normal animals treated with DYN 12 (cross-hatched bar). Data are expressed in kilopascals (kPA).

Figure 15 is a graphic illustration of the effects of DYN 12 (3-O-methylsorbitollysine) treatment on skin elasticity. Diabetic or normal rats were treated with DYN 12 (50 mg/kg daily) or saline for eight weeks and then subjected to skin elasticity tests. The four groups used included diabetic controls (saline injection; solid black bar), diabetics treated with DYN 12 (open bar), normal animal controls (saline injections; stippled bar), and normal animals treated with DYN 12 (cross-hatched bar). Data are expressed in kilopascals (kPA) and are shown as averages of the results obtained with each particular group of test subjects. Measurements were taken on the hind leg of the test subjects and were taken on an alert animal restrained by a technician.

Figure 16 is a schematic illustration of a novel metabolic pathway in the kidney. The formation of 3DG in the kidney occurs using either endogenous glycated protein or glycated protein derived from dietary sources. By way of the endogenous pathway, the chemical combination of glucose and lysine leads to glycated protein. Alternatively, glycated protein may also be obtained from dietary sources. Catabolism of glycated proteins results in the production of fructoselysine, which is subsequently acted upon by Amadorase. Amadorase, a fructosamine-3-kinase, is part of both pathways. Amadorase phosphorylates fructoselysine to form fructoselysine-3-phosphate, which may then be converted to 3-deoxyglucosone (3DG), producing byproducts of lysine and inorganic phosphate (A very small amount of fructoselysine (<5% total fructoselysine) may be converted to 3DG by way of a non-enzymatic pathway). 3DG may then be detoxified by conversion to 3-deoxyfructose (3DF) or it may go on to produce reactive oxygen species (ROS) and advanced glycation end products (AGEs). As shown in Figure 16, DYN 12 (3-O-methylsorbitollysine) inhibits the action of Amadorase on fructoselysine, and DYN 100 (arginine) inhibits the 3DG-mediated production of ROS and AGEs.
Figure 17 is a schematic illustration of the disease states affected by reactive oxygen species (ROS). 3DG may produce ROS directly, or it may produce advanced glycation end products which go on to form ROS. The ROS are then responsible for advancing various disease states as shown in the figure.

Figure 18 is a schematic illustration of both adduct formation and inhibition of adduct formation according to embodiments of the present invention. 3DG can form an adduct with a primary amino group on a protein. Protein-3DG adduct formation creates a Schiff base, the equilibrium of which is depicted in Figure 18. The protein-3DG Schiff base adduct may go on to form a crosslinked protein, by formation of a second protein-3DG adduct by way of the 3DG molecule involved in the first protein-3DG Schiff base adduct described above, thereby forming a "3DG bridge" between two primary amino groups of a single protein (pathway "A").

Alternatively, such crosslinking may occur between two primary amino groups of separate proteins, forming a "3DG bridge" between two primary amino groups of two separate proteins, resulting in a crosslinked pair of protein molecules. The first protein-3DG Schiff base adduct may be prevented from going on to form such crosslinked proteins as depicted in pathway "A." For example, such protein crosslinking may be inhibited by nucleophilic agents such as glutathione or penicillamine, as illustrated in Figure 18 by pathway "B." Such nucleophilic agents react with the 3DG carbon atom responsible for forming the second Schiff base, preventing that carbon atom from forming a Schiff base protein-3DG adduct and thereby preventing crosslinking of the protein.

Figure 19 is a graph depicting a standard curve for phosphatase in a phosphate assay as described herein.

Figure 20 is a graph depicting the linearity of a phosphate assay as described herein.

Figure 21, comprising Figures 21A-21G, is a series of images depicting compounds of the invention which are useful in methods of the present invention, including inhibition of fructosamine-3-kinase.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates generally to compositions and methods of treating deleterious conditions that involve inhibiting the production or effect of
alpha-dicarbonyl sugars such as 3DG in the affected tissue and/or removing the sugars from the affected tissue. This is because it has now been discovered, as described in greater detail elsewhere herein, that removal of underlying causative factors of the deleterious conditions results in amelioration of the deleterious conditions. Such deleterious conditions include, but are not limited to, inflammation, pain and itch.

The invention also relates to the novel discovery, set forth herein for the first time, that compositions comprising both an inhibitor of alpha-dicarbonyl sugar formation and an inhibitor of alpha-dicarbonyl sugar function or effect, together exhibit a synergistic effect in the alleviation of alpha-dicarbonyl sugar-associated conditions, as compared with compositions comprising either type of inhibitor alone. One particularly advantageous combination is the combination of meglumine and arginine for the treatment of alpha-dicarbonyl sugar-associated conditions.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described herein.

As used herein, each of the following terms has the meaning associated with it in this section.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

The term "accumulation of 3DG" or "accumulation of alpha-dicarbonyl sugars" as used herein refers to an detectable increase in the level of 3DG and/or alpha-dicarbonyl sugar overtime.

"Alpha-dicarbonyl sugar," as used herein, refers to a family of compounds, including 3-Deoxyglucosone, glyoxal, methyl glyoxal and glucosone.

"Alpha-dicarbonyl sugar associated parameter of wrinkling, aging, disease or disorder of the skin," as used herein, refers to the biological markers described herein, including 3DG levels, 3DF levels, fructosamine kinase levels,
protein crosslinking, and other markers or parameters associated with alpha-dicarbonyl sugar associated wrinkling, aging, diseases or disorders of the skin.

"3-Deoxyglucosone" or "3DG," as used herein, refers to the 1,2-dicarbonyl-3-deoxysugar (also known as 3-deoxyhexulosone), which can be formed via an enzymatic pathway or can be formed via a nonenzymatic pathway. For purposes of the present description, the term 3-deoxyglucosone is an alpha-dicarbonyl sugar which can be formed by pathways including the nonenzymatic pathway described in Figure 1 and the enzymatic pathway resulting in breakdown of FL3P described in Figure 2. Another source of 3DG is diet. 3DG is a member of the alpha-dicarbonyl sugar family, also known as 2-oxoaldehydes.

A "3DG associated" or "3DG related" disease or disorder as used herein, refers to a disease, condition, or disorder which is caused by indicated by or associated with 3DG, including defects related to enhanced synthesis, production, formation, and accumulation of 3DG, as well as those caused by medicated by or associated with decreased levels of degradation, detoxification, binding, and clearance of 3DG.

"A 3DG inhibiting amount" or an "alpha-dicarbonyl inhibiting amount" of a compound refers to that amount of compound which is sufficient to inhibit the function or process of interest, such as synthesis, formation accumulation and/or function of 3DG or another alpha-dicarbonyl sugar.

"3-O-methyl sorbitolylsine (3-O-Me-sorbitolylsine)," is an inhibitor of fructosamine kinases, as described herein. It is used interchangeably with the term "DYN 12".

As used herein, "alleviating a disease or disorder symptom," means reducing the severity of the symptom.

The term "AGE-proteins" (Advanced Glycation End product modified proteins), as used herein, refers to a product of the reaction between sugars and proteins (Brownlee, 1992, Diabetes Care, 15: 1835; Niwa et al., 1995, Nephron, 69: 438. For example, the reaction between protein lysine residues and glucose, which does not stop with the formation of fructose-lysine (FL). FL can undergo multiple dehydration and rearrangement reactions to produce non-enzymatic 3DG, which reacts again with free amino groups, leading to cross-linking and browning of the
protein involved. AGEs also include the products that form from the reaction of 3DG with other compounds, such as, but not limited to, as shown in Figure 16.

"Amadorase," as used herein, refers to a fructosamine kinase responsible for the production of 3-DG. More specifically it refers to a protein which can enzymatically convert fructoselysine (FL) to fructoselysine-3-phosphate (FL3P), as defined above, when additionally supplied with a source of high energy phosphate.

"F3K," as used herein, refers to fructosamine-3-kinase, which is one type of fructosamine kinase. A non-limiting example of a reaction catalyzed by F3K is the conversion of FL to FL3P.

The term "Amadori product," as used herein, refers to a ketoamide, such as, but not limited to, fructoselysine, comprising is a rearrangement product following glucose interaction with the ε-NH2 groups of lysine-containing proteins.

As used herein, "amino acids" are represented by the full name thereof, by the three-letter code corresponding thereto, or by the one-letter code corresponding thereto, as indicated in the following table:

<table>
<thead>
<tr>
<th>Full Name</th>
<th>Three-Letter Code</th>
<th>One-Letter Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic Acid</td>
<td>Asp</td>
<td>D</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>Glu</td>
<td>E</td>
</tr>
<tr>
<td>Lysine</td>
<td>Lys</td>
<td>K</td>
</tr>
<tr>
<td>Arginine</td>
<td>Arg</td>
<td>R</td>
</tr>
<tr>
<td>Histidine</td>
<td>His</td>
<td>H</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyr</td>
<td>Y</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Cys</td>
<td>C</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Asn</td>
<td>N</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Gln</td>
<td>Q</td>
</tr>
<tr>
<td>Serine</td>
<td>Ser</td>
<td>S</td>
</tr>
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<td>Threonine</td>
<td>Thr</td>
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<td>Valine</td>
<td>Val</td>
<td>V</td>
</tr>
<tr>
<td>Leucine</td>
<td>Leu</td>
<td>L</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>He</td>
<td>I</td>
</tr>
<tr>
<td>Methionine</td>
<td>Met</td>
<td>M</td>
</tr>
</tbody>
</table>
Proline                   Pro                   P
Phenylalanine            Phe                   F
Tryptophan               Tip                   W

The term "binding" refers to the adherence of molecules to one another, such as, but not limited to, enzymes to substrates, ligands to receptors, antibodies to antigens, DNA binding domains of proteins to DNA, and DNA or RNA strands to complementary strands.

"Binding partner," as used herein, refers to a molecule capable of binding to another molecule.

The term "biological sample," as used herein, refers to samples obtained from a living organism, including skin, hair, tissue, blood, plasma, cells, sweat and urine.

The term "clearance," as used herein refers to the physiological process of removing a compound or molecule, such as by diffusion, exfoliation, removal via the bloodstream, and excretion in urine, or via other sweat or other fluid.

A "coding region" of a gene consists of the nucleotide residues of the coding strand of the gene and the nucleotides of the non-coding strand of the gene which are homologous with or complementary to, respectively, the coding region of an mRNA molecule which is produced by transcription of the gene.

"Complementary" as used herein refers to the broad concept of subunit sequence complementarity between two nucleic acids, e.g., two DNA molecules. When a nucleotide position in both of the molecules is occupied by nucleotides normally capable of base pairing with each other, then the nucleic acids are considered to be complementary to each other at this position. Thus, two nucleic acids are complementary to each other when a substantial number (at least 50%) of corresponding positions in each of the molecules are occupied by nucleotides which normally base pair with each other (e.g., A:T and G:C nucleotide pairs). Thus, it is known that an adenine residue of a first nucleic acid region is capable of forming specific hydrogen bonds ("base pairing") with a residue of a second nucleic acid region which is antiparallel to the first region if the residue is thymine or uracil.

Similarly, it is known that a cytosine residue of a first nucleic acid strand is capable of base pairing with a residue of a second nucleic acid strand which is antiparallel to the first strand if the residue is guanine. A first region of a nucleic acid is complementary
to a second region of the same or a different nucleic acid if, when the two regions are arranged in an antiparallel fashion, at least one nucleotide residue of the first region is capable of base pairing with a residue of the second region. Preferably, the first region comprises a first portion and the second region comprises a second portion, whereby, when the first and second portions are arranged in an antiparallel fashion, at least about 50%, and preferably at least about 75%, at least about 90%, or at least about 95% of the nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion. More preferably, all nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion.

A "compound," as used herein, refers to any type of substance or agent that is commonly considered a drug, or a candidate for use as a drug, as well as combinations and mixtures of the above, or modified versions or derivatives of the compound.

As used herein, the terms "conservative variation" or "conservative substitution" refer to the replacement of an amino acid residue by another, biologically similar residue. Conservative variations or substitutions are not likely to significantly change the shape of the peptide chain. Examples of conservative variations, or substitutions, include the replacement of one hydrophobic residue such as isoleucine, valine, leucine or alanine for another, or the substitution of one charged amino acid for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like.

"Detoxification" of 3DG refers to the breakdown or conversion of 3DG to a form which does not allow it to perform its normal function. Detoxification can be brought about or stimulated by any composition or method, including "pharmacologic detoxification", or metabolic pathway which can cause detoxification of 3DG.

"Pharmacologic detoxification of "3DG" or other alpha-dicarbonyl sugars refers to a process in which a compound binds with or modifies 3DG, which in turn causes it to be become inactive or to be removed by metabolic processes such as, but not limited to, excretion.
A "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate. As used herein, normal aging is included as a disease.

A "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

As used herein, the term "domain" refers to a part of a molecule or structure that shares common physicochemical features, such as, but not limited to, hydrophobic, polar, globular and helical domains or properties such as ligand binding, signal transduction, cell penetration and the like. Specific examples of binding domains include, but are not limited to, DNA binding domains and ATP binding domains.

An "effective amount" or "therapeutically effective amount" of a compound is that amount of compound which is sufficient to provide a beneficial effect to the subject to which the compound is administered, or gives the appearance of providing a therapeutic effect as in a cosmetic.

As used herein, the term "effector domain" refers to a domain capable of directly interacting with an effector molecule, chemical, or structure in the cytoplasm which is capable of regulating a biochemical pathway.

"Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA. Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino
acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

The term "floating," as used herein, refers to bonds of a substituent to a ring structure, such that the substituent can be attached to the ring structure at any available carbon juncture. A "fixed" bond means that a substituent is attached at a specific site.

The term "formation of 3DG" refers to 3DG which is not necessarily formed via a synthetic pathway, but can be formed via a pathway such as spontaneous or induced breakdown of a precursor.

As used herein, the term "fragment," as applied to a protein or peptide, can ordinarily be at least about 3-15 amino acids in length, at least about 15-25 amino acids, at least about 25-50 amino acids in length, at least about 50-75 amino acids in length, at least about 75-100 amino acids in length, and greater than 100 amino acids in length.

As used herein, the term "fragment," as applied to a nucleic acid, can ordinarily be at least about 20 nucleotides in length, typically, at least about 50 nucleotides, more typically, from about 50 to about 100 nucleotides, preferably, at least about 100 to about 200 nucleotides, even more preferably, at least about 200 nucleotides to about 300 nucleotides, yet even more preferably, at least about 300 to about 350, even more preferably, at least about 350 nucleotides to about 500 nucleotides, yet even more preferably, at least about 500 to about 600, even more preferably, at least about 600 nucleotides to about 620 nucleotides, yet even more preferably, at least about 620 to about 650, and most preferably, the nucleic acid fragment will be greater than about 650 nucleotides in length.

The term "fructose-lysine" (FL) is used herein to signify any glycated-lysine, whether incorporated in a protein/peptide or released from a protein/peptide by proteolytic digestion. This term is specifically not limited to the chemical structure commonly referred to as fructose-lysine, which is reported to form from the reaction of protein lysine residues and glucose. As noted above, lysine amino groups can react with a wide variety of sugars. Indeed, one report indicates that glucose is the least reactive sugar out of a group of sixteen (16) different sugars tested (Bunn et al., Science, 213: 222 (1981)). Thus, tagatose-lysine formed from galactose and lysine, analogously to glucose is included wherever the term fructose-lysine is mentioned in
this description, as is the condensation product of all other sugars, whether naturally-occuring or not. It will be understood from the description herein that the reaction between protein-lysine residues and sugars involves multiple reaction steps. The final steps in this reaction sequence involve the crosslinking of proteins and the production of multimeric species, known as AGE-proteins, some of which are fluorescent. Once an AGE protein forms, then proteolytic digestion of such AGE-proteins does not yield lysine covalently linked to a sugar molecule. Thus, these species are not included within the meaning of "fructose-lysine", as that term is used herein.

The term "Fructose-lysine-3-phosphate," as used herein, refers to a compound formed by the enzymatic transfer of a high energy phosphate group from ATP to FL. The term fructose-lysine-3-phosphate (FL3P), as used herein, is meant to include all phosphorylated fructose-lysine moieties that can be enzymatically formed whether free or protein-bound.

"Fructose-lysine-3-phosphate kinase" (FL3K), as used herein, refers to one or more proteins, such as amadorase, which can enzymatically convert FL to FL3P, as described herein, when supplied with a source of high energy phosphate. The term is used interchangeably with "fructose-lysine kinase (FLK)" and with "amadorase".

"Fructose-lysine-3-phosphate kinase activity," as used herein, refers to the enzymatic conversion of FL to FL3P.

"Fructosamine-3-phosphate kinase activity," as used herein, refers to the enzymatic conversion of fructose to fructose-3-phosphate (F3P). For example, fructosamine-3-phosphate kinase activity generally encompasses the conversion of fructose, or a fructose derivative, to fructose-3-phosphate, or the corresponding fructose derivative-3-phosphate. A fructose derivative includes, but is not limited to, fructose lysine.

The term "FL3P Lysine Recovery Pathway," as used herein, refers to a lysine recovery pathway which exists in human skin and kidney, and possibly other tissues, and which regenerates unmodified lysine as a free amino acid or as incorporated in a polypeptide chain.

The term "Glycated Diet," as used herein, refers to any given diet in which a percentage of normal protein is replaced with glycated protein. The
expressions "glycated diet" and "glycated protein diet" are used interchangeably herein.

"Glycated lysine residues," as used herein, refers to the modified lysine residue of a stable adduct produced by the reaction of a reducing sugar and a lysine-containing protein.

The majority of protein lysine residues are located on the surface of proteins as expected for a positively charged amino acid. Thus, lysine residues on proteins, which come in contact with serum, or other biological fluids, can freely react with sugar molecules in solution. This reaction occurs in multiple stages. The initial stage involves the formation of a Schiff base between the lysine free amino group and the sugar keto-group. This initial product then undergoes the Amadori rearrangement, to produce a stable ketoamine compound.

This series of reactions can occur with various sugars. When the sugar involved is glucose, the initial Schiff base product will involve imine formation between the aldehyde moiety on C-I of the glucose and the lysine ε-amino group. The Amadori rearrangement will result in formation of lysine coupled to the C-I carbon of fructose, 1-deoxy-l-(ε-aminolysine)-fructose, herein referred to as fructose-lysine or FL. Similar reactions will occur with other aldose sugars, for example galactose and ribose (Dills, 1993, Am. J. Clin. Nutr. 58:S779). For the purpose of the present invention, the early products of the reaction of any reducing sugar and the ε-amino residue of protein lysine are included within the meaning of glycated-lysine residue, regardless of the exact structure of the modifying sugar molecule.

As used herein, "homologous" or homology" are used synonymously with "identity". The determination of percent identity or homology between two nucleotide or amino acid sequences can be accomplished using a mathematical algorithm. For example, a mathematical algorithm useful for comparing two sequences is the algorithm of Karlin and Altschul (1990, Proc. Natl. Acad. Sci. USA 87:2264-2268), modified as in Karlin and Altschul (1993, Proc. Natl. Acad. Sci. USA 90:5873-5877). This algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990, J. Mol. Biol. 215:403-410), and can be accessed, for example at the National Center for Biotechnology Information (NCBI) world wide web site. BLAST nucleotide searches can be performed with the NBLAST program (designated "blastn" at the NCBI web site), using the following parameters: gap
penalty = 5; gap extension penalty = 2; mismatch penalty = 3; match reward = 1;
expectation value 10.0; and word size = 11 to obtain nucleotide sequences
homologous to a nucleic acid described herein. BLAST protein searches can be
performed with the XBLAST program (designated "blastn" at the NCBI web site) or
the NCBI "blastp" program, using the following parameters: expectation value 10.0,
BLOSUM62 scoring matrix to obtain amino acid sequences homologous to a protein
molecule described herein. To obtain gapped alignments for comparison purposes,
Gapped BLAST can be utilized as described in Altschul et al. (1997, Nucleic Acids
Res. 25:3389-3402). Alternatively, PSI-Blast or PHI-Blast can be used to perform an
iterated search which detects distant relationships between molecules (Id.) and
relationships between molecules which share a common pattern. When utilizing
BLAST, Gapped BLAST, PSI-Blast, and PHI-Blast programs, the default parameters
of the respective programs (e.g., XBLAST and NBLAST) can be used.

The percent identity between two sequences can be determined using
techniques similar to those described above, with or without allowing gaps. In
calculating percent identity, typically exact matches are counted. The term "induction
of 3DG" or "inducing 3DG," as used herein, refers to methods or means which start
or stimulate a pathway or event leading to the synthesis, production, or formation of
3DG or increase in its levels, or stimulate an increase in function of 3DG. Similarly,
the phrase "induction of alpha-dicarbonyl sugars", refers to induction of members of
the alpha-dicarbonyl sugar family, including 3DG, glyoxal, methyl glyoxal, and
glucosone.

"Inhibiting 3DG" as described herein, refers to any method or
technique which inhibits 3DG synthesis, production, formation, accumulation, or
function, as well as methods of inhibiting the induction or stimulation of synthesis,
formation, accumulation, or function of 3DG. It also refers to any metabolic pathway
which can regulate 3DG function or induction. The term also refers to any
composition or method for inhibiting 3DG function by detoxifying 3DG or causing
the clearance of 3DG. Inhibition can be direct or indirect. Induction refers to
induction of synthesis of 3DG or to induction of function. Similarly, the phrase
"inhibiting alpha-dicarbonyl sugars", refers to inhibiting members of the alpha-
dicarbonyl sugar family, including 3DG, glyoxal, methyl glyoxal, and glucosone.
The term "inhibiting accumulation of 3DG," as used herein, refers to the use of any composition or method which decreases synthesis, increases degradation, or increases clearance, of 3DG such that the result is lower levels of 3DG or functional 3DG in the tissue being examined or treated, compared with the levels in tissue not treated with the composition or method. Similarly, the phrase "inhibiting accumulation of alpha-dicarbonyl sugars", refers to inhibiting accumulation of members of the alpha-dicarbonyl sugar family, including 3DG, glyoxal, methyl glyoxal, and glucosone, and intermediates thereof.

As used herein, an "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the peptide of the invention in the kit for effecting alleviation of the various diseases or disorders recited herein. Optionally, or alternately, the instructional material can describe one or more methods of alleviating the diseases or disorders in a cell or a tissue of a mammal. The instructional material of the kit of the invention can, for example, be affixed to a container which contains the identified compound invention or be shipped together with a container which contains the identified compound. Alternatively, the instructional material can be shipped separately from the container with the intention that the instructional material and the compound be used cooperatively by the recipient.

An "isolated nucleic acid" refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring state, e.g., a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, e.g., the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, e.g., RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g, as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence. "Modified" compound, as used herein, refers to a modification or
derivation of a compound, which may be a chemical modification, such as in chemically altering a compound in order to increase or change its functional ability or activity.

The term "mutagenicity" refers to the ability of a compound to induce or increase the frequency of mutation. The term "nucleic acid" typically refers to large polynucleotides.

The term "oligonucleotide" typically refers to short polynucleotides, generally, no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequences (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T."

The term "peptide" typically refers to short polypeptides.

"Permeation enhancement" and "permeation enhancers" as used herein relate to the process and added materials which bring about an increase in the permeability of skin to a poorly skin permeating pharmacologically active agent, i.e., so as to increase the rate at which the drug permeates through the skin and enters the bloodstream. "Permeation enhancer" is used interchangeably with "penetration enhancer".

As used herein, the term "pharmaceutically-acceptable carrier" means a chemical composition with which an appropriate compound or derivative can be combined and which, following the combination, can be used to administer the appropriate compound to a subject.

As used herein, the term "physiologically acceptable" ester or salt means an ester or salt form of the active ingredient which is compatible with any other ingredients of the pharmaceutical composition, which is not deleterious to the subject to which the composition is to be administered.

"Polypeptide" refers to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof linked via peptide bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof.

A "polynucleotide" means a single strand or parallel and anti-parallel strands of a nucleic acid. Thus, a polynucleotide may be either a single-stranded or a double-stranded nucleic acid.
"Primer" refers to a polynucleotide that is capable of specifically hybridizing to a designated polynucleotide template and providing a point of initiation for synthesis of a complementary polynucleotide. Such synthesis occurs when the polynucleotide primer is placed under conditions in which synthesis is induced, i.e., in the presence of nucleotides, a complementary polynucleotide template, and an agent for polymerization such as DNA polymerase. A primer is typically single-stranded, but may be double-stranded. Primers are typically deoxyribonucleic acids, but a wide variety of synthetic and naturally occurring primers are useful for many applications. A primer is complementary to the template to which it is designed to hybridize to serve as a site for the initiation of synthesis, but need not reflect the exact sequence of the template. In such a case, specific hybridization of the primer to the template depends on the stringency of the hybridization conditions. Primers can be labeled with, e.g., chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

As used herein, the term "promoter/regulatory sequence" means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulator sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

A "constitutive" promoter is a promoter which drives expression of a gene to which it is operably linked, in a constant manner in a cell. By way of example, promoters which drive expression of cellular housekeeping genes are considered to be constitutive promoters.

An "inducible" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living cell substantially only when an inducer which corresponds to the promoter is present in the cell.

A "tissue-specific" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.
A "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

The term "protein" typically refers to large polypeptides.

Reactive Oxygen Species Various harmful forms of oxygen are generated in the body; singlet oxygen, superoxide radicals, hydrogen peroxide, and hydroxyl radicals all cause tissue damage. A catchall term for these and similar oxygen related species is "reactive oxygen species" (ROS). The term also includes ROS formed by the internalization of AGEs into cells and the ROS that form therefrom.

"Removing 3-deoxyglucosone," as used herein, refers to any composition or method, the use of which results in lower levels of 3-deoxyglucosone (3DG) or lower levels of functional 3DG when compared to the level of 3DG or the level of functional 3DG in the absence of the composition. Lower levels of 3DG can result from its decreased synthesis or formation, increased degradation, increased clearance, or any combination of thereof. Lower levels of functional 3DG can result from modifying the 3DG molecule such that it can function less efficient in the process of glycation or can result from binding of 3DG with another molecule which blocks inhibits the ability of 3DG to function. Lower levels of 3DG can also result from increased clearance and excretion in urine of 3DG. The term is also used interchangeably with "inhibiting accumulation of 3DG". Similarly, the phrase "removing alpha-dicarbonyl sugars", refers to removal of members of the alpha-dicarbonyl sugar family, including 3DG, glyoxal, methyl glyoxal, and glucosone.

Also, the terms glycated-lysine residue, glycated protein and glycosylated protein or lysine residue are used interchangeably herein, is consistently with current usage in the art where such terms are art-recognized used interchangeably.

The term "skin," as used herein, refers to the commonly used definition of skin, e.g., the epidermis and dermis, and the cells, glands, mucosa and connective tissue which comprise the skin.

The term "standard," as used herein, refers to something used for comparison. For example, it can be a known standard agent or compound which is...
administered and used for comparing results when administering a test compound, or it can be a standard parameter or function which is measured to obtain a control value when measuring an effect of an agent or compound on a parameter or function. "Standard" can also refer to an "internal standard", such as an agent or compound which is added at known amounts to a sample and which is useful in determining such things as purification or recovery rates when a sample is processed or subjected to purification or extraction procedures before a marker of interest is measured. Internal standards are often but are not limited to, a purified marker of interest which has been labeled, such as with a radioactive isotope, allowing it to be distinguished from an endogenous substance in a sample.

A "susceptible test animal," as used herein, refers to a strain of laboratory animal which, due to for instance the presence of certain genetic mutations, have a higher propensity toward a disease disorder or condition of choice, such as diabetes, cancer, and the like.

"Synthesis of 3DG", as used herein refers to the formation or production of 3DG. 3DG can be formed based on an enzyme dependent pathway or a non-enzyme dependent pathway. Similarly, the phrase "synthesis of alpha-dicarbonyl sugars", refers to synthesis or spontaneous formation of members of the alpha-dicarbonyl sugar family, including 3DG, glyoxal, methyl glyoxal, and glucosone, and adducts as disclosed herein.

"Synthetic peptides or polypeptides" mean a non-naturally occurring peptide or polypeptide. Synthetic peptides or polypeptides can be synthesized, for example, using an automated polypeptide synthesizer. Those of skill in the art know of various solid phase peptide synthesis methods.

A "therapeutic" treatment is a treatment administered to a subject who exhibits signs of pathology, for the purpose of diminishing or eliminating those signs.

By "transdermal" delivery is intended both transdermal (or "percutaneous") and transmucosal administration, i.e., delivery by passage of a drug through the skin or mucosal tissue and into the bloodstream. Transdermal also refers to the skin as a portal for the administration of drugs or compounds by topical application of the drug or compound thereto.
The term "topical application", as used herein, refers to administration to a surface, such as the skin. This term is used interchangeably with "cutaneous application".

The term to "treat," as used herein, means reducing the frequency with which symptoms are experienced by a patient or subject or administering an agent or compound to reduce the frequency with which symptoms are experienced.

As used herein, "treating a disease or disorder" means reducing the frequency with which a symptom of the disease or disorder is experienced by a patient. Disease and disorder are used interchangeably herein.

As used herein, the term "wild-type" refers to the genotype and phenotype that is characteristic of most of the members of a species occurring naturally and contrasting with the genotype and phenotype of a mutant.

In accordance with the present invention, it has been determined that a composition containing an inhibitor of 3DG production and an inhibitor of 3DG function was able to down-regulate allograft inflammatory factor I (AIF-I) in cultured lymphocytes. AIP-I is one of the first inflammatory proteins expressed in transplantation rejection and is believed to be involved in the inflammation observed in graft-versus-host disease. AIF-I is also believed to be an important inflammatory molecule in the pathogenesis of systemic sclerosis (scleroderma). Indeed, AIF-I is believed to function in a diverse array of inflammatory responses.

Accordingly, the compositions and methods of the present invention are expected to find utility in the treatment of a wide variety of diseases and disorders in which inflammation plays a role. These include, among others, allergic conditions, Alzheimer's disease, anemia, angiogenesis, aortic valve stenosis, arthritis, atherosclerosis, thrombosis, rheumatoid arthritis, osteoarthritis, gout, gouty arthritis, acute pseudogout, acute gouty arthritis, inflammation associated with cancer, congestive heart failure, cystitis, fibromyalgia, fibrosis, glomerulonephritis, inflammation associated with gastro-intestinal disease, inflammatory bowel diseases, kidney failure, glomerulonephritis, myocardial infarction, ocular diseases, pancreatitis, psoriasis, reperfusion injury or damage, respiratory disorders, restenosis, septic shock, inflammatory conditions of the skin, endotoxic shock, urosepsis, stroke, surgical complications, systemic lupus erythematosus, transplantation associated
arteriopathy, graft vs. host reaction, allograft rejection, chronic transplant rejection and vasculitis.

In accordance with particular aspects of the present invention, it has been demonstrated that topical application of composition containing an inhibitor of 3DG production and an inhibitor of 3DG function resulted in decreased redness and irritation associated with razor burn. A topical formulation comprising the same active agents was reported by participants in a skin irritation trial to decrease redness associated with detergent chapping, to accelerate the healing process, and to cause an overall improvement in skin texture as compared with a formulation that did not contain the active agents. In addition, topical application of that composition has been found to decrease inflammation associated with psoriasis, eczema and polycythemia, and to decrease the number and severity of facial acne lesions.

In view of the disclosure set forth herein, inflammatory conditions of the skin are considered particularly amenable to treatment by targeting alpha-dicarbonyl sugar production and function. Inflammatory conditions of the skin contemplated for treatment in accordance with embodiments of the present invention include, but are not limited to: transient inflammation and irritation of skin due to hair removal by shaving, waxing, tweezing, electrolysis, or use of depilatory products; various forms of dermatitis, including seborrheic dermatitis, nummular dermatitis, contact dermatitis, atopic dermatitis, exfoliative dermatitis, perioral dermatitis and stasis dermatitis, to name some common examples; and inflammatory skin diseases or disorders such as psoriasis, folliculitis, rosacea, telangiectasia, acne, impetigo, erysipelas, paronychia, erythrasma, eczema, rash (diaper rash, poison ivy, poison oak) and sunburn, to name a few.

Also as set forth in the present disclosure, topical application of a composition containing an inhibitor of 3DG production and an inhibitor of 3DG function resulted in decreased pain associated with sinus inflammation. The same formulation was also reported to provide relief from joint swelling, pain and tenderness in arthritic patients when topically applied to the skin overlying the affected joint tissue.

In one aspect of the invention, inflammatory conditions of tissues underlying the skin are also considered particularly amenable to treatment by targeting alpha-dicarbonyl sugar production and function. Inflammatory conditions of
underlying tissues include, but are not limited to: sinus pressure and inflammation; joint tissue inflammation associated with various forms of arthritic disease, such as rheumatoid arthritis, osteoarthritis, gout, gouty arthritis, acute pseudogout and acute gouty arthritis.

Methods of Inhibiting Synthesis, Formation, and Accumulation of 3DG and Other Alpha-dicarbonyl Sugars

It has been discovered in the present invention that an enzyme which is involved in the enzymatic synthetic pathway of 3DG production is present at high levels in skin (see Example 20). Furthermore, it has also been discovered in the present invention that 3DG is present at high levels in skin (see Example 19). Accordingly, the invention includes compositions and methods which interfere with both enzymatic and nonenzymatic based synthesis or formation of 3DG in skin, and which also interfere with the function of 3DG in skin. 3DG is a member of a family of compounds called alpha-dicarbonyl sugars. Other members of the family include glyoxal, methyl glyoxal, and glucosone. The present invention also relates to compositions and methods for inhibiting accumulation of 3DG and other alpha-dicarbonyl sugars in skin and for inhibiting 3DG dependent or associated skin wrinkling, skin aging, or other skin diseases or disorders, as well as skin wrinkling, skin aging, or other skin diseases and disorders associated with other alpha-dicarbonyl sugars. The invention also includes inhibiting accumulation of 3DG in skin using compositions and methods for stimulating the pathways, or components of the pathways, leading to 3DG detoxification, degradation, or clearance from the skin.

It should be noted that 3DG is a member of the alpha-dicarbonyl sugar family of molecules. It should also be noted that other members of the alpha-dicarbonyl sugar family can perform functions similar to 3DG, as described herein, and that like 3DG functions, the functions of other members of the alpha-dicarbonyl sugar family are inhibitable as well. Thus, the invention should be construed to include methods of inhibiting synthesis, formation, and accumulation of other alpha-dicarbonyl sugars as well.

Inhibition of 3DG synthesis, formation, and accumulation in skin can be direct or indirect. For example, direct inhibition of 3DG synthesis refers to blocking an event that occurs immediately prior to or upstream in a pathway of 3DG
synthesis or formation, such as blocking amadorase or the conversion of fructose-
lysine-3-phosphate (FL3P) to 3DG, lysine, and inorganic phosphate. Indirect
inhibition can include blocking or inhibiting upstream precursors, enzymes, or
pathways, which lead to the synthesis of 3DG. Components of an upstream pathway,
for example, include the amadorase gene and amadorase mRNA. The invention
should not be construed to include inhibition of only the enzymatic and nonenzymatic
pathways described herein, but should be construed to include methods of inhibiting
other enzymatic and nonenzymatic pathways of 3DG synthesis, formation and
accumulation in skin as well. The invention should also be construed to include the
other members of the alpha-dicarbonyl sugar family, including glyoxal, methyl
glyoxal, and glucosone where applicable.

Various assays described herein may be used to directly measure 3DG
synthesis or levels of 3DG, or assays maybe used which are correlative of 3DG
synthesis or levels, such as measurement of its breakdown product, 3DF.

The present invention includes novel methods for the inhibition of
3DG synthesis in skin. Preferably, the skin is mammalian skin, and more preferably,
the mammal skin is human skin.

In one aspect, the inhibitor inhibits an enzyme involved in the
synthesis of 3DG. In one embodiment the enzyme is a fructosamine kinase. In yet
another embodiment the fructosamine kinase is amadorase, as disclosed in U.S. Patent
No 6,004,958.

In one embodiment of the invention, the inhibitor inhibits the
accumulation of 3DG in the skin. In one aspect, the 3DG is synthesized or formed in
the skin. However, the inhibitor can also inhibit accumulation of 3DG in the skin,
where the source of 3DG is other than the skin. In one aspect, the source of the 3DG
is dietary, i.e., it is derived from an external source rather than an internal source, and
then accumulates in the skin. Thus, this aspect of the invention includes the inhibition
of 3DG synthesis or formation in the skin and/or inhibition of accumulation of 3DG in
the skin. In the latter case, the source of 3DG may be enzymatic synthesis of 3DG
directly in the skin, enzymatic synthesis of 3DG in a tissue other than skin,
nonenzymatic synthesis or formation of 3DG in the skin or in a non-skin tissue, or the
source of the 3DG may be external, such as, for example, dietary. The methods to be
used for inhibiting accumulation of 3DG or other alpha-dicarbonyl sugars via any one of these pathways are more fully described elsewhere herein.

The present invention also relates to methods and compositions for treating tissues other than skin. As described in detail elsewhere herein, and as will be understood by the skilled artisan when armed with the present disclosure, the methods and compositions of the invention are equally applicable to any tissue in which 3DG exists and can exist. Such tissues include, but are not limited to, kidney and pancreas. Therefore, the compositions and methods of the invention will be understood to be equally applicable to tissues that contain or can contain 3DG.

In another embodiment of the invention, a method of inhibiting the synthesis, formation, or accumulation of 3DG in the skin, and in other tissues, is useful to prevent inflammation. As set forth in detail elsewhere herein, inhibition of synthesis, formation or accumulation of 3DG contributes to inflammation and inflammatory processes. Therefore, the present invention features a method of diminishing or inhibiting inflammation by inhibiting the synthesis, formation and/or accumulation of 3DG.

In yet another embodiment of the invention, a method is provided for the inhibition of inflammation by inhibiting allograft inflammatory factor-1 (AIF-I). As set forth in detail elsewhere herein, AIF-I plays a role in a diverse array of inflammatory and inflammation-related processes, including, but not limited to, scleroderma and graft-versus-host disease. As disclosed for the first time herein, methods and compositions of the invention can inhibit the activity and/or function of AIF-I, thereby decreasing or preventing the inflammatory processes to which AIF-I contributes. That is, compositions and methods of the invention can be used to treat a patient with an inflammatory condition related to ADF-I.

In one embodiment, the invention provides a method of using a composition useful for alleviating or preventing an inflammatory condition in a patient, wherein the condition is related to AIF-I. In one aspect, a composition comprises an N-methyl-glucamine-like compound. In a preferred aspect, the compound is meglumine. In another aspect of the invention, a composition comprises meglumine and arginine. In yet another aspect of the invention, a composition useful for inhibiting AIF-I or AIF-I activity is fructoselysine. It will be understood by the skilled artisan, when armed with the disclosure set forth herein, that a useful
compound of the invention (e.g., meglumine, arginine) can be altered or modified in
order to increase or decrease the activity of the compound against a desired target of
the invention, such as AIF-I. Modifications are described elsewhere herein, and can
be made according to methods known in the art.

In another embodiment of the invention, a method is provided of using
a composition of the invention for the treatment of inflammation or of an
inflammation-related condition in a mammal, wherein the inflammatory condition is
associated with one or more major organs in the mammal. In one aspect, the mammal
is a human. Major organs include, for example, skin, heart, eyes, kidneys, pancreas,
lungs, and the circulatory system. In another aspect, a composition of the invention is
provided in an oral dosage form to a mammal. Such compositions useful in a method
according to the invention are described in detail elsewhere herein. By way of a non-
limiting example, such compositions include meglumine and meglumine + arginine.

In yet another embodiment of the invention, compositions and methods
are provided for the treatment of pain in a mammal. Pain is a complicated process
that involves interplay between a number of important chemicals, called
neurotransmitters, that transmit nerve impulses from one nerve cell to another. There
are many different neurotransmitters in the human body, and, in the case of pain, act
in various combinations to produce painful sensations in the body. Some chemicals
govern mild pain sensations; others control intense or severe pain.

The body's chemicals act in the transmission of pain messages by
stimulating neurotransmitter receptors found on the surface of cells; each receptor has
a corresponding neurotransmitter. Receptors function much like gates or ports and
enable pain messages to pass through and on to neighboring cells. One brain chemical
of special interest to neuroscientists is glutamate. During experiments, mice with
blocked glutamate receptors show a reduction in their responses to pain. Other
important receptors in pain transmission are opiate-like receptors. Morphine and other
opioid drugs work by locking on to these opioid receptors, switching on pain-
inhibiting pathways or circuits, and thereby blocking pain.

Another type of receptor that responds to painful stimuli is called a
nociceptor. Nociceptors are thin nerve fibers in the skin, muscle, and other body
tissues, that, when stimulated, carry pain signals to the spinal cord and brain.
Normally, nociceptors only respond to strong physical stimuli. However, when tissues
become injured or inflamed, they release chemicals that make nociceptors much more sensitive and cause them to transmit pain signals in response to even gentle stimuli. This condition is called alldynia, a state in which pain is produced by innocuous stimuli.

It has been shown herein for the first time that compositions and methods, as set forth herein, are useful to diminish or alleviate pain in a mammal. In one aspect, the mammal is a human. Such a method comprises administering a composition of the invention to a mammal, either topically or orally. Compositions useful in a method of alleviating or diminishing pain according to the present invention are described in detail elsewhere herein. By way of a non-limiting example, such compositions include meglumine and meglumine + arginine. Other compositions useful in methods of the invention are set forth elsewhere herein in greater detail.

Various types of pain treatable by the compositions and methods, as set forth herein, include arachnoiditis; arthritis, such as osteoarthritis, and rheumatoid arthritis; ankylosing spondylitis; gout; tendonitis; bursitis sciatica; spondylolisthesis; radiculopathy; burn pain; cancer pain; headaches; migraines; cluster headaches; and tension headaches; trigeminal neuralgia; myofascial pain; neuropathic pain, including diabetic neuropathy, reflex sympathetic dystrophy syndrome, phantom limb and post-amputation pain; tendonitis; tenosynovitis; postherpetic neuralgia; shingles-associated pain; central pain syndrome; trauma-associated pain; vasculitis; pain associated with infections, including herpes simplex; skin tumors, cysts; and tumors associated with neurofibromatosis; and pain associated with strains, bruises, dislocations; fractures; and pain due to exposure to chemicals (e.g. exfoliants such as retinoids, carboxylic acids, beta-hydroxy acids, alpha-keto acids, benzoyl peroxide and phenol).

In still another embodiment of the invention, compositions and methods are provided for the treatment of itch in a mammal. In origin, itch can be cutaneous ("pruritoceptive", e.g. dermatitis), neuropathic (e.g. multiple sclerosis), neurogenic (e.g. cholestasis), mixed (e.g. uraemia) or psychogenic. Although itch of cutaneous origin shares a common neural pathway with pain, the afferent C-fibres subserving itch are a functionally distinct subset: they respond to histamine, acetylcholine and other pruritogens, but are insensitive to mechanical stimuli.
Different types of itch have responded to various treatments. Histamine is the main mediator for itch in insect bite reactions and in most forms of urticaria, and in these circumstances the itch responds well to H1-antihistamines. However, in most dermatoses and in systemic disease, low-sedative H1-antihistamines are ineffective. Opioid antagonists relieve itch caused by spinal opioids, cholestasis and, possibly, uraemia. Ondansetron relieves itch caused by spinal opioids (but not cholestasis and uraemia). Other drug treatments for itch include rifampicin, colestyramine and 17-alkyl androgens (cholestasis), thalidomide (uraemia), cimetidine and corticosteroids (Hodgkin's lymphoma), paroxetine (paraneoplastic itch), aspirin and paroxetine (polycythaemia vera) and indometacin (some HIV+ patients). Ultraviolet B therapy, particularly narrow-band UVB, has been postulated as a treatment for itch in uraemia. This is because it has been shown herein for the first time that compositions and methods, as set forth herein, are useful to diminish or alleviate itch in a mammal. In one aspect, the mammal is a human. Such a method comprises administering a composition of the invention to a mammal, either topically or orally. Compositions useful in a method of alleviating or diminishing itch according to the present invention are described in detail elsewhere herein. By way of a non-limiting example, such compositions include meglumine and meglumine + arginine. Other compositions useful in methods of the invention are set forth elsewhere herein in greater detail.

In another embodiment, the present invention provides a method for treatment of inflammation, itch, pain, and other diseases or disorders as set forth herein, as well as those that will be apparent from the disclosure, wherein the treatment is by way of a composition comprising two or more compounds, further wherein the combination of compounds results in a synergistic effect of treatment. That is, the result of the treatment with the combination of compounds is greater than the additive effect of the results of treatment with each compound separately.

In one embodiment of the invention, a method of treating a patient includes treatment with a composition comprising both an inhibitor of alpha-dicarbonyl sugar formation and an inhibitor of alpha-dicarbonyl sugar function or effect, wherein the multiple inhibitors together exhibit a synergistic effect in the alleviation of alpha-dicarbonyl sugar-associated conditions, as compared with compositions comprising either type of inhibitor alone. In a preferred embodiment, a
method includes the combination of meglumine and arginine for the treatment of alpha-dicarbonyl sugar-associated conditions.

While not wishing to be limited by any particular theory, it is noted that arginine not only inactivates 3DG, as set forth in detail elsewhere herein, but arginine also feeds into the nitric oxide pathway and stimulates NO production which causes vasodilation. This complements the anti-oxidative, anti-inflammatory action of meglumine so the effect of meglumine and arginine in combination is greater than the additive effect of treatment with each compound alone. Other compositions useful in methods of the invention are set forth elsewhere herein in greater detail, and such compounds may also be combined with arginine to obtain a synergistic effect for treatment.

In another aspect of the invention, compositions and methods are provided for the treatment of kidney-related diseases and disorders, such as, but not limited to, uremia and azotemia. In one aspect of the invention, a composition is provided to treat uremia in a patient, wherein the composition inhibits the production of 3DG. In another aspect, a composition is provided to treat uremia in a patient, wherein the composition inhibits the function of 3DG. In yet another aspect, a composition is provided to treat uremia in a patient, wherein the composition inhibits the production and function of 3DG. It will be understood that such a composition may be comprised of one or more components, and that any component in such a composition may individually have properties of inhibiting the production of 3DG, inhibiting the function of 3DG, or both.

In another aspect of the invention, a method is provided to treat uremia in a patient, wherein the method inhibits the production of 3DG. In another aspect, a method is provided to treat uremia in a patient, wherein the method inhibits the function of 3DG. In yet another aspect, a method is provided to treat uremia in a patient, wherein the method inhibits the production and function of 3DG. It will be understood that such a method may be useful for inhibiting the production of 3DG, inhibiting the function of 3DG, or both.

Methods of Treating Diabetes
The invention also relates to compositions and methods for treating diabetes. Diabetes, and in particular, type II diabetes, is associated with damage to
the pancreas. Type II diabetes results from a combination of genetic and lifestyle factors. In people genetically predisposed to diabetes, overeating and lack of physical activity lead to insulin resistance with characteristic postprandial hyperglycemia. Obesity is an inflammatory disease characterized by elevated levels of the proinflammatory cytokines TNF-alpha, IL-6 and IL-1, all of which contribute to insulin resistance (rev in Wellen, K.E. and Hotamisligil, G.S. 2005. J. Clin. Invest. 115:1111-1119). In the pre-diabetic BB rat, there are elevated levels of allograft inflammatory factor 1 (AIF) in the pancreas (Chen Z.-W. et al. 1997. PNAS 94:13897-13884). Together, the inflammatory state, elevated lipid levels and oxidative stress state characteristic of 'metabolic syndrome' leads to diminished pancreatic function due to beta cell apoptosis, resulting in Type II diabetes. This condition may be further exacerbated in that diabetics also have increased levels of 3DG, which also leads to release of cytokines, production of inflammatory advanced glycation endproducts (AGEs) and increased oxidative stress.

Therefore, the present invention provides compositions and methods for treating diabetes. In one embodiment, the invention provides a method comprising administering to a patient a composition as set forth in detail elsewhere herein, wherein the composition alleviates the diabetic condition of the patient. In another embodiment, a method includes administration to a patient a composition as set forth in detail herein, wherein the composition prevents a diabetic condition in a patient predisposed to diabetes. Compositions useful for treating diabetes are described in detail elsewhere herein in greater detail. Examples of such compositions include, but should not be limited to, meglumine and meglumine + arginine. Other compositions useful in methods of the invention are set forth elsewhere herein in greater detail.

Especially since the pancreas has elevated levels of F3K enzyme activity, produces elevated levels of fructose lysine 3 phosphate which breaks down into 3DG. Hence the pancreas is making its own 3DG which has an effect locally to destroy beta cells and adversely effect supporting extracellular matrix and vascularization of the pancreas.

Methods of Removing 3DG from Skin
The present invention also relates to compositions and methods for removing 3DG and other alpha-dicarbonyl sugars from skin and for inhibiting 3DG dependent or associated skin wrinkling, skin aging, or other skin diseases or disorders, as well as skin wrinkling, skin aging, or other skin diseases and disorders associated with other alpha-dicarbonyl sugars. To this end, the invention includes compositions and methods for inhibiting the production, synthesis, formation, and accumulation of 3DG in skin. The invention also includes compositions and methods for stimulating the pathways, or components of the pathways, leading to 3DG detoxification, degradation, or clearance from the skin.

**Compounds and Methods to Inhibit F3K**

In one embodiment the invention includes a method of inhibiting 3DG synthesis in the skin of a mammal, said method comprising administering to a mammal an effective amount of an inhibitor of 3DG synthesis, or a derivative or modification thereof, thereby inhibiting 3DG synthesis in the skin of a mammal. Preferably, the mammal is a human.

In one embodiment, the inhibitor comprises from about 0.0001% to about 15% by weight of the pharmaceutical composition. In one aspect, the inhibitor is administered as a controlled-release formulation. In another aspect the pharmaceutical composition comprises a lotion, a cream, a gel, a liniment, an ointment, a paste, a toothpaste, a mouthwash, an oral rinse, a coating, a solution, a powder, and a suspension. In yet another aspect, the composition further comprises a moisturizer, a humectant, a demulcent, oil, water, an emulsifier, a thickener, a thinner, a surface active agent, a fragrance, a preservative, an antioxidant, a hydrotropic agent, a chelating agent, a vitamin, a mineral, a permeation enhancer, a cosmetic adjuvant, a bleaching agent, a depigmentation agent, a foaming agent, a conditioner, a viscosifier, a buffering agent, and a sunscreen.

The invention should be construed to include various methods of administration, including topical, oral, intramuscular, and intravenous.

In one aspect of the invention, the inhibitor of 3DG synthesis is an inhibitor of fructosamine kinase/amadorase. In one aspect, the fructosamine kinase is F3K. The inhibitor of fructosamine kinase can be a compound such as N-methyl-
glucamine and N-methyl-glucamine-like compounds. In one embodiment of the invention, an inhibitor of 3DG synthesis is meglumine.

In another embodiment of the invention, a F3K inhibitor is not meglumine. In one embodiment, an inhibitor of 3DG synthesis is not meglumine. In one aspect, an F3K inhibitor is any F3K inhibitor other than meglumine. In yet another aspect, an F3K inhibitor is selected from a limited group of inhibitors, wherein the group of inhibitors does not comprise meglumine.

In one aspect of the invention, representative inhibitor compounds having the above formula include galactitol lysine, 3-deoxy sorbitol lysine, 3-deoxy-3-fluoro-xylitol lysine, and 3-deoxy-3-cyano sorbitol lysine and 3-O-methyl sorbitol lysine. Examples of known compounds that may be used as inhibitors in practicing this invention include, without limitation, meglumine, sorbitol lysine, galactitol lysine, and mannitol lysine. Other compositions useful as inhibitors are set forth elsewhere herein in greater detail.

The compounds of the invention may be administered to, for example, a cell, a tissue, or a subject by any of several methods described herein and by others which are known to those of skill in the art. In one aspect, an inhibitor of the invention which inhibits enzymatic synthesis of 3DG may be synthesized in vitro using techniques known in the art (see Example 8).

In another aspect of the invention, a compound useful in the invention, and useful in a method of the invention, is an inhibitor of a fructosamine kinase. In an embodiment, a compound is an inhibitor of fructosamine-3-kinase.

In another embodiment, a fructosamine-3-kinase inhibitor of the invention is selected from:

(1) a compound of formula I, or a salt thereof:

![Chemical Structure](image-url)
wherein:

Ar is independently selected at each occurrence from the group consisting of
aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with
one or more substituents, independently selected from halogen; (Ci-C(_6))alkyl;
(Ci-C(_6))alkenyl; (Ci-C(_6))alkoxy; OH; NO_2; ON; C(O)O(Ci-C_3)alkyl;
(C_2-C_6)alkylene-OR; phosphonato; NR_2; NHC(=O)(Ci-C_6)alkyl; sulfamyl;
carbamyl; OC(=O)(C_2-C_3)alkyl; O(C_2-C_6)alkylene-N((Ci-C_6)alkyl)_2; and
(Ci-C_3)perfluoroalkyl; and

R^2 is independently selected at each occurrence from the group consisting of
hydrogen and (Ci-C_6)alkyl;

(2) a compound of formula II, or a salt thereof:

![II](image)

wherein

Ar is independently selected at each occurrence from the group consisting of
aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with
one or more substituents, independently selected from halogen; (Ci-Ce)alkyl;
(Ci-C_6)alkenyl; (Ci-C_6)alkoxy; OH; NO_2; CN; C(=O)O(Ci-C_3)alkyl;
(C_2-C_6)alkylene-OR; phosphonato; NR_2; NHC(=O)(Ci-C_6)alkyl; sulfamyl;
carbamyl; OC(=O)(C_2-C_3)alkyl; O(C_2-C_6)alkylene-N((Ci-C_6)alkyl)_2; and
(Ci-C_3)perfluoroalkyl;

G^1 is independently selected at each occurrence from the group consisting of
C=O and CH_2, provided that at least one occurrence of G^1 is C=O;
-L- is selected from the group consisting of -NH-C(=O)-, -C(=O)-NH-, -O-, -S-, and -NR_2-;

R^1 independently selected at each occurrence from the group consisting of
hydrogen; halogen; (C_1-C_6)alkyl; (C_1-C_6)alkenyl; (C_1-CeOaIkOXy; OH; NO_2;
CN; C(=O)O(Ci-C_3)alkyl; (C_2-C_6)alkylene-OR; phosphonato; NR_2;
NHC(=O)(C\textsubscript{i-C\textsubscript{6}})alkyl; sulfamyl; carbamyl; OC(=O)(C\textsubscript{r-C\textsubscript{3}})alkyl; O(C\textsubscript{2-C\textsubscript{6}})alkylene-N((C\textsubscript{i-C\textsubscript{6}})alkyl)\textsubscript{2}; and (C\textsubscript{1-C\textsubscript{3}})perfluoroalkyl; and

R\textsubscript{2} is independently selected at each occurrence from the group consisting of hydrogen and (C\textsubscript{i-C\textsubscript{6}})alkyl;

(3) a compound of formula III, or a salt thereof:

![Chemical Structure](attachment:image.png)

wherein

G\textsubscript{2} is selected from the group consisting of formulae III\textsubscript{1}, III\textsubscript{2}, and III\textsubscript{3}:

![Chemical Structures](attachment:image.png)

G\textsubscript{3} is selected from the group consisting of NR\textsubscript{2}, C(R\textsubscript{2})\textsubscript{2}, O\textsubscript{3} and S;

G\textsubscript{4} is C(R\textsubscript{3})\textsubscript{2}; and

Ar is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; (C\textsubscript{i-C\textsubscript{g}})alkyl; (C\textsubscript{i-C\textsubscript{6}})alkenyl; (C\textsubscript{r-C\textsubscript{6}})alkoxy; OH; NO\textsubscript{2}; C=\textsubscript{N}; C(=O)O(C\textsubscript{r-C\textsubscript{3}})alkyl; (C\textsubscript{2-C\textsubscript{6}})alkylene-OR\textsubscript{2}; phosphonato; NR\textsubscript{2}; NHC(=O)(C\textsubscript{i-C\textsubscript{6}})alkyl; sulfamyl; carbamyl; OC(=O)(C\textsubscript{r-C\textsubscript{3}})alkyl; O(C\textsubscript{2-C\textsubscript{6}})alkylene-N((C\textsubscript{i-C\textsubscript{6}})alkyl)\textsubscript{2}; and (C\textsubscript{1-C\textsubscript{3}})perfluoroalkyl;

R\textsubscript{2} independently selected at each occurrence from the group consisting of hydrogen and (C\textsubscript{i-C\textsubscript{6}})alkyl;

m is 2 or 3; and
n is 1, 2, or 3;

(4) a compound of formula IV, or a salt thereof:

![Chemical Structure](image)

IV

wherein

G\(_3\) is selected from the group consisting of NR\(_2\), C(R\(^2\))\(_2\), O, and S;

G\(_5\) is independently selected at each occurrence from the group consisting of NR\(_2\), O, and S;

G\(_6\) is selected from the group consisting of Ar, Ar-((Ci-C\(_6\))alkylene), and formula IV\(^1\):

![Chemical Structure](image)

IV\(^1\)

G\(_4\) is C(R\(^3\))\(_2\);

G\(_7\) is selected from the group consisting of Ar and Ar-((Ci-C\(_6\))alkylene);

Ar is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; (Ci-Ce)alkyl; (Ci-C\(_6\))alkenyl; (C\(_1\)-C\(_6\))alkoxy; OH; NO\(_2\); C=N; C(=O)O(C\(_1\)-C\(_3\))alkyl; (C\(_2\)-C\(_6\))alkylene-OR\(^2\); phosphonato; NR\(^2\); NHC(=O)(Ci-C\(_6\))alkyl; sulfamyl; carbamyl; OC(=O)(Ci-C\(_3\))alkyl; O(C\(_2\)-C\(_6\))alkylene-N((Ci-C\(_6\))alkyl)\(_2\); and (Ci-C\(_3\))perfluoroalkyl; and

R\(^2\) independently selected at each occurrence from the group consisting of hydrogen and (Ci-Ce)alkyl;
(5) a compound of formula V, or a salt thereof:

wherein

G\textsuperscript{3} is selected from the group consisting of NR\textsuperscript{2}, C(R\textsuperscript{2})\textsubscript{2}, O, and S;

G\textsuperscript{5} is independently selected at each occurrence from the group consisting of NR\textsuperscript{2}, O, and S;

G\textsuperscript{7} is independently selected at each occurrence from the group consisting of Ar, and Ar-((Ci-C\textsubscript{6})\textsubscript{6}alkylene);

Ar is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; (Ci-C\textsubscript{6})alkyl; (Ci-C\textsubscript{6})alkenyl; (C\textsubscript{-}C\textsubscript{6})alkoxy; OH; NO\textsubscript{2}; C\equiv N; C(=O)O(C\textsubscript{-}C\textsubscript{3})alkyl; (C\textsubscript{2}-C\textsubscript{6})alkylene-OR\textsuperscript{2}; phosphonato; NR\textsuperscript{2}; NHC(=O)(Ci-C\textsubscript{6})alkyl; sulfamyl; carbamyl; OC(=O)(Ci-C\textsubscript{3})alkyl; O(C\textsubscript{2}-C\textsubscript{6})alkylene-N((Ci-C\textsubscript{6})alkyl)\textsubscript{2}; and (Ci-C\textsubscript{6})perfluoroalkyl; and

R\textsuperscript{2} independently selected at each occurrence from the group consisting of hydrogen and (Ci-C\textsubscript{6})alkyl;

(6) a compound of formula VI, or a salt thereof:

wherein
G⁵ is selected from the group consisting of NR², O, and S;

G⁶ is selected from the group consisting of Ar, Ar-((Ci-C₆)alkylene) and formula V⁰¹:

G⁴ is C(R₃)₂;

G⁷ is selected from the group consisting of Ar and Ar-((Ci-C₆)alkylene);

G⁸ is N or CR¹;

Ar is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; (Ci-C₆)alkyl; (Ci-C₆)alkenyl; (Cᵢ-Cᵢ)alkoxy; OH; NO₂; C≡N; C(=O)O(Cᵢ-Cᵢ)alkyl; (C₂-C₆)alkylene-OR²; phosphonato; NR²; NHC(=O)(Ci-C₆)alkyl; sulfamyl; carbamyl; OC(=O)(d-C₃)alkyl; O(C₂-C₆)alkylene-N(((Ci-C₆)alkyl)₂; and (Ci-C₃)perfluoroalkyl; and

R¹ independently selected at each occurrence from the group consisting of hydrogen; halogen; (Ci-C₆)alkyl; (Ci-C₆)alkenyl; (Ci-C₆)alkoxy; OH; NO₂; C≡N; C(=O)O(Cᵢ-Cᵢ)alkyl; (C₂-C₆)alkylene-OR²; phosphonato; NR²; NHC(=O)(Ci-C₆)alkyl; sulfamyl; carbamyl; OC(=O)(Cᵢ-C₆)alkyl; O(C₂-C₆)alkylene-N(((Ci-C₆)alkyl)₂; and (Ci-C₃)perfluoroalkyl; and

R² independently selected at each occurrence from the group consisting of hydrogen and (Ci-C₆)alkyl.

(7) a compound of formula VII, or a salt thereof:
VIII

wherein

G^8 is N or CR;  
G^9 is O or S;

Ar is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; (Ci-C6)alkyl; (Ci-C6)alkenyl; (Ci-C6)alkoxy; OH; NO2; C≡N; C(=O)O(Ci-C3)alkyl; (C2-C6)alkylene-OR2; phosphonato; NR2; NHC(O)(Ci-C6)alkyl; sulfamyl; carbamyl; OC(O)(Ci-C3)alkyl; O(C2-C6)alkylene-N((Ci-C6)alkyl)2; and (C1-C3)perfluoroalkyl; and

R^1 independently selected at each occurrence from the group consisting of hydrogen; halogen; (d-C6)alkyl; (C1-C6)alkenyl; (d-C6)alkoxy; OH; NO2; C≡N; C(=O)O(Ci-C3)alkyl; (C2-C6)alkylene-OR2; phosphonato; NR2; NHC(O)(Ci-C6)alkyl; sulfamyl; carbamyl; OC(O)(Ci-C3)alkyl; O(C2-C6)alkylene-N((Ci-C6)alkyl)2; and (Ci-C6)perfluoroalkyl; and

R^2 independently selected at each occurrence from the group consisting of hydrogen and (Ci-C6)alkyl.

(8) a compound of formula VIII, or a salt thereof:

\[
\begin{align*}
G^8 \quad & \quad G^9 \\
\end{align*}
\]

wherein

G^{10} is independently selected at each occurrence from the group consisting of formulae VIII^1, VIII^2, and VIII^3, VIII^4, and VIII^5:
R³ is independently selected at each occurrence from the group consisting of Hydrogen, -OH, -CH₂OH, and -CH₃, and G¹ı provided that G¹ı may be selected no more than once for each occurrence of VIII¹, VIII², VIII³, VIII⁴, or VIII⁵;

G¹ı is independently selected at each occurrence from the group consisting of formulae VIII⁶, VIII⁷, VIII⁸, VIII⁹, and VIII¹⁰:

R⁴ is independently selected at each occurrence from the group consisting of Hydrogen, -OH, -CH₂OH, and -CH₃.

(9) a compound of formula IX or X, or a salt thereof:
wherein

$R^5$ is independently selected at each occurrence from the group consisting of

Hydrogen; F; Cl; Br; I; (d-C$_6$)alkyl; (C$_6$-C$_8$)alkenyl; (C$_6$-C$_9$)alkoxy; OH; NO$_2$; C$_5$N; C(=O)(C$_6$-C$_9$)alkyl; (C$_2$C$_6$)alkylene-OR$_2$; phosphonato; NR$_2$; NHC(=O)(C$_6$-C$_9$)alkyl; sulfamyl; carbamyl; OC(=O)(C$_2$-C$_3$)alkyl; O(C$_2$-C$_6$)alkylene-N((C$_6$-C$_9$)alkyl)$_2$; and (C$_6$-C$_9$)perfluoroalkyl;

$R^2$ independently selected at each occurrence from the group consisting of

hydrogen and (C$_6$-C$_9$)alkyl;

Ar is independently selected at each occurrence from the group consisting of

aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; (C$_6$-C$_9$)alkyl; (C$_r$C$_6$)alkenyl; (C$_r$C$_6$)alkoxy; OH; NO$_2$; C$_5$N; C(=O)(C$_r$C$_3$)alkyl; (C$_2$C$_6$)alkylene-OR$_2$; phosphonato; NR$_2$; NHC(=O)(C$_6$-C$_9$)alkyl; sulfamyl; carbamyl; OC(=O)(C$_2$-C$_3$)alkyl; O(C$_2$-C$_6$)alkylene-N((C$_6$-C$_9$)alkyl)$_2$; and (C$_6$-C$_9$)perfluoroalkyl;

In the definitions of each of the compounds of formulae I to X above:

the term "aryl", employed alone or in combination with other terms, means,

unless otherwise stated, a carbocyclic aromatic system containing one or more rings (typically one, two or three rings) wherein such rings may be attached together in a pendant manner, such as a biphenyl, or may be fused, such as naphthalene. Examples include phenyl; anthracyl; and naphthyl. Preferred are

phenyl and naphthyl, most preferred is phenyl.

the term "heteroaryl" refers to a heterocycle having aromatic character. Examples of heteroaryl groups include: pyridyl, pyrazinyl, pyrimidinyl, particularly 2 and 4pyrimidinyl, pyridazinyl, thi-enyl, furyl, pyrrolyl, particularly 2pyrrolyl, imidazolyl, thiazolyl, oxazolyl, pyrazolyl, particularly 3 and 5pyrazolyl, iso-thiazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,3,4-triazolyl, tetrazolyl, 1,2,3-thiadiazolyl, 1,2,3-oxadiazolyl, 1,3,4-thiadiazolyl and 1,3,4-oxadiazolyl. A polycyclic heteroaryl may include one or more rings
which are partially saturated, such as tetrahydroquinoline and 2,3-dihydrobenzofuryl. Examples of polycyclic heterocycles include: indolyl, particularly 3-, 4-, 5-, 6- and 7-indolyl, indolinyl, quinolyl, tetrahydroquinolyl, isoquinolyl, particularly 1- and 5-isoquinolyl, 1,2,3,4-tetrahydroisoquinolyl, cinnolinyl, quinoxalinyl, particularly 2- and 5-quinoxalinyl, quinazolinyl, phthalazinyl, 1,8-naphthyridinyl, 1,4-benzodioxanyl, coumarin, dihydrocoumarin, benzofuryl, particularly 3-, 4-, 1,5-naphthyridinyl, 5-, 6-, and 7-benzofuryl, 2,3-dihydrobenzofuryl, 1,2-benzisoxazolyl, benzothienyl, particularly 3-, 4-, 5-, and 7-benzothiienyl, benzoxazolyl, benzthiazolyl, particularly 2-benzothiazolyl and 5-benzothiazolyl, purinyl, benzimidazolyl, particularly 2-benzimidazolyl, benztriazolyl, thioxanthinyl, carbazolyl, carboliny1, acridinyl, pyrrolidinyl, and quinolizidinyl.

the term "alkyl", by itself or as part of another substituent means, unless otherwise stated, a straight, branched or cyclic chain hydrocarbon having the number of carbon atoms designated (i.e. C<sub>i</sub>-C<sub>6</sub> means one to six carbons) and includes straight, branched chain or cyclic groups. Examples include: methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tertbutyl, pentyl, neopentyl, hexyl, cyclohexyl and cyclopropylmethyl. Most preferred is (C<sub>i</sub>-C<sub>3</sub>)alkyl, particularly ethyl, methyl and isopropyl.

the term "alkenyl" employed alone or in combination with other terms, means, unless otherwise stated, a stable monounsaturated or diunsaturated straight chain, branched chain or cyclic hydrocarbon group having the stated number of carbon atoms. Examples include vinyl, propenyl (allyl), crotyl, isopentenyl, butadienyl, 1,3-pentadienyl, 1,4-pentadienyl, cyclopentenyl, cyclopentadienyl and the higher homologs and isomers. A functional group representing an alkene is exemplified by CH=CHCH<sub>2</sub>.

the term "alkylene", by itself or as part of another substituent means, unless otherwise stated, a divalent straight, branched or cyclic chain hydrocarbon.

the term "alkoxy" employed alone or in combination with other terms means, unless otherwise stated, an alkyl group having the designated number of carbon atoms, as defined above, connected to the rest of the molecule via an oxygen atom, such as, for example, methoxy, ethoxy, 1-propano1, 2-propano1.
(isopropoxy) and the higher homologs and isomers. Preferred are
(Ci-C₃)alkoxy, particularly ethoxy and methoxy.

the term halogen means, unless otherwise stated, a fluorine, chlorine, bromine,
or iodine atom, preferably, fluorine, chlorine, or bromine, more preferably,
fluorine or chlorine.

the term "(Cₓ-Cᵧ)perfluoroalkyl," wherein x < y, means an alkyl group with a
minimum of x carbon atoms and a maximum of y carbon atoms, wherein all
hydrogen atoms are replaced by fluorine atoms. Preferred is
(Ci-C₆)perfluoroalkyl, more preferred is (Ci-C₃)perfluoroalkyl, most preferred
is -CF₃.

In another embodiment of the invention, a fructosamine-3-kinase
inhibitor is a compound of any one of formulae I to X wherein Ar is selected from the
group consisting of phenyl or thiophenyl, said phenyl or thiophenyl optionally
substituted with one or two substituents selected from the group consisting of
halogen, preferably chlorine or fluorine; (Ci-Cₑ)alkyl, preferably methyl;
Ci-C₆)alkoxy, preferably methoxy, OH, and NHC(=O)(Ci-C₆)alkyl.

In another embodiment, a compound of any one of formulae I to X
includes a compound wherein R² is hydrogen, methyl, ethyl, or isopropyl;

In another embodiment, a fructosamine-3-kinase inhibitor is a
compound of formula IA, or a salt thereof:

IA

wherein Ar is as defined above for formula I;

more preferably, the compound of formula IB, or a salt thereof:
In another embodiment, a fructosamine-3-kinase inhibitor is a compound of formula HA, or a salt thereof:

\[
\text{HA}
\]

wherein Ar, G1, and L are as defined above for formula II;
more preferably, a compound of formula HB, or a salt thereof:

\[
\text{HB}
\]

wherein Ar and L are as defined above for formula II;
more preferably, a compound of formula HC, or a salt thereof:

\[
\text{HC}
\]

wherein Ar is as defined above for formula II;
yet more preferably, a compound of formula HD or a salt thereof:
wherein Alk is (Ci-Ce)alkyl;
most preferably, the compound of formula HE or a salt thereof:

5

In another embodiment, a fructosamine-3-kinase inhibitor is a compound of formula HIA, or a salt thereof:

10

wherein Ar, G^2, G^3, and G^4 are as defined above for formula III;
more preferably a compound of formula IHB, or a salt thereof:

15

wherein Ar, and G^2 are as defined above for formula III;
yet more preferably a compound of formula iπc, or a salt thereof:
wherein Ar is as defined above for formula III;
most preferably a compound of formula HID, or a salt thereof:

![HID](image)

In another embodiment, a fructosamine-3-kinase inhibitor is a compound of formula IVA, or a salt thereof:

![IVA](image)

wherein Ar, G₃, and G₆ are as defined above for formula IV;
more preferably a compound of formula IVB, or a salt thereof:

![IVB](image)
IVB

wherein $G^7$ is as defined above for formula IV;
most preferably, a compound of formula IVC or IVD, or a salt thereof.

![IVC](image)

![IVD](image)

In another embodiment, a fructosamine-3-kinase inhibitor is a compound of formula VA, or a salt thereof:

![VA](image)

wherein $R^2$ and $G^7$ are as defined above for formula V;
more preferably a compound of formula VB, or a salt thereof:
wherein $R^2$ and $A_r$ are as defined above for formula V;
most preferably, the compound of formula VC, or a salt thereof:

In another embodiment, a fructosamine-3-kinase inhibitor is a compound of formula VIA, or a salt thereof:

wherein $A_r$, $R^1$, and $G^6$ are as defined above for formula VI;
more preferably, a compound of formula VIB, or a salt thereof
wherein Ar is as defined above for formula VI; most preferably, a compound of formula VIC, or a salt thereof.

In another embodiment, a fructosamine-3-kinase inhibitor is a compound of formula VIIA, or a salt thereof.

wherein G⁸, G⁹, R¹, and Ar are as defined for formula VII; more preferably, a compound of formula VIIB, or a salt thereof.
wherein \(G^9, R^1,\) and \(Ar\) are as defined for formula VII; most preferably, a compound of formula VIIC, or a salt thereof.

In another embodiment, a fructosamine-3-kinase inhibitor is a compound of formula VIII A, or a salt thereof:
In another embodiment, a fructosamine-3-kinase inhibitor is a compound of any one of formulae IXA-IXZ, as well as IXAA and IXAB, or a salt thereof:

- IXA
- IXB
- IXC
- IXD
- IXE
- IXF
In another embodiment, a fructosamine-3-kinase inhibitor is a compound of formula XA, or a salt thereof:

\[ \text{IXV} \]

\[ \text{IXW} \]

\[ \text{IXX} \]

\[ \text{IXY} \]

\[ \text{IXZ} \]

\[ \text{IXAA} \]

\[ \text{IXAB} \]

In an embodiment, the compounds of I to X may be made by a method comprising the combination of benzidine and a saccharide in a Raney™ nickel reaction. By way of a non-limiting example, a compound of formula VIII may be made by a method comprising dissolving 1 mmol benzidine and 2 mmol 6-deoxyglucose in 100 ml of ethanol-water (1:1), and adding RanNi (100 mg). The suspension is shaken in a Parr hydrogenator at 50 psi of hydrogen. When the reaction is complete, the catalyst is Filtered and the filtrate concentrated to induce crystallization of product.

It will be understood that when compounds of formulae I to X contain one or more chiral centers, the compounds may exist in, and may be isolated as pure enantiomeric or diastereomeric forms or as racemic mixtures. The present invention therefore includes any possible enantiomers, diastereomers, racemates or mixtures.
thereof of the compounds of the invention which have the property of inhibiting fructosamine-3-kinase activity.

The isomers resulting from the presence of a chiral center comprise a pair of non superimposable isomers that are called "enantiomers." Single enantiomers of a pure compound are optically active, i.e., they are capable of rotating the plane of plane polarized light.

The present invention is meant to encompass diastereomers as well as their racemic and resolved, diastereomerically and enantiomerically pure forms and salts thereof. Diastereomeric pairs may be resolved by known separation techniques including normal and reverse phase chromatography, and crystallization.

By "isolated optical isomer" is meant a compound which has been substantially purified from the corresponding optical isomer(s) of the same formula. Preferably, the isolated isomer is at least about 80%, more preferably at least 90% pure, even more preferably at least 98% pure, most preferably at least about 99% pure, by weight.

Isolated optical isomers may be purified from racemic mixtures by well known chiral separation techniques. According to one such method, a racemic mixture of a compound having the structure of one of Formulae I to X, or a chiral intermediate thereof, is separated into 99% wt.% pure optical isomers by HPLC using a suitable chiral column, such as a member of the series of DAICEL® CHIRALPAK® family of columns (Daicel Chemical Industries, Ltd., Tokyo, Japan). The column is operated according to the manufacturer's instructions, but the skilled artisan will understand how such operation can be modified depending upon particular needs and desired results of the chromatographic step.

Where permitted by their structure the compounds of formulae I to X may take the form of salts. The term "salts," embraces addition salts of free acids or free bases which are compounds of the invention. The term "pharmaceutically-acceptable salt" refers, in part, to salts which possess toxicity profiles within a range that affords utility in pharmaceutical applications.

Suitable pharmaceutically-acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of inorganic acids include hydrochloric, hydrobromic, hydriodic, nitric, carbonic, sulfuric, and
phosphoric acids. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which include formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, antranilic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, trifluoromethanesulfonic, 2-hydroxyethanesulfonic, p-toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, alginic, \( \beta \)-hydroxybutyric, salicylic, galactic and galacturonic acid. Examples of pharmaceutically unacceptable acid addition salts include, for example, perchlorates and tetrafluoroborates.

Suitable pharmaceutically acceptable base addition salts of compounds of the invention include for example, metallic salts including alkali metal, alkaline earth metal and transition metal salts such as, for example, calcium, magnesium, potassium, sodium and zinc salts. Pharmaceutically acceptable base addition salts also include organic salts made from basic amines such as, for example, \( \text{N} \)-dibenzylethlenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Examples of pharmaceutically unacceptable base addition salts include lithium salts and cyanate salts.

All of these salts may be prepared by conventional means from the compounds of Formula I to \( X \) by reacting, for example, the appropriate acid or base with the compounds according to Formula I to \( X \).

The invention, as disclosed herein, also relates to the involvement of 3DG in causing various skin diseases and disorders and to methods of inhibiting the function of 3DG in order to alleviate or treat 3DG associated skin diseases and disorders. The invention also relates to the involvement of 3DG in other diseases and disorders, such as gum diseases and disorders. Such gingival diseases and disorders include, but are not limited to, gingivitis, receding gums, and other 3DG or other alpha-dicarboxyl sugar associated gingival diseases and disorders. As described above, inhibition of 3DG function can be direct or indirect. Therefore, 3DG function may be inhibited or caused to decrease using many approaches as described herein. Inhibition of 3DG function may be assayed or monitored using techniques described herein as well as others known to those of skill in the art. Function can be measured
directly or it can be estimated using techniques to measure parameters which are known to be correlative of 3DG function. For example, protein crosslinking and protein production can be measured directly using techniques such as electrophoretic analysis (see Figure 12 and Examples 7 and 18) as well as other techniques (see Examples 21-24). The invention should be construed to include not only compounds useful for preventing 3DG induced crosslinking of molecules such as collagen, elastin, and proteoglycans, but it should also be construed to include compounds which inhibit crosslinking of other molecules as well. The invention should also be construed to include the use of compounds to modulate other 3DG functions as well, such as apoptosis and formation of reactive oxygen species. It is known that in macrophage-derived cells apoptotic cell death can be induced by methylglyoxal and 3DG (Okado et al., 1996, Biochem. Biophys. Res. Commun. 225:219-224). In yet another aspect of the invention, an inhibitor of 3DG inhibits an active oxygen species (Vander Jagt et al., 1997, Biochem. Pharmacol. 53: 1133-1140). The invention should be construed to include other alpha-dicarbonyl sugars as well. 3DG and its detoxification product 3DF can be measured several ways using cell, tissue, blood, plasma, and urine samples (see Examples 4, 5, 6, 14, 15, and 17) and FL, a product produced during the synthesis of 3DG, can also be measured (see Examples 5), as can a precursor, FL3P (see Figures 1 and 2 and Examples 1, 2, and 3).

The invention discloses methods which are useful for inhibiting 3DG function in the skin. Such a method includes administering an effective amount of one or more inhibitors of 3DG function, or modifications or derivatives thereof, in a pharmaceutical composition to a subject.

In one aspect of the invention the 3DG function inhibitor inhibits protein crosslinking. In another aspect, the inhibitor inhibits formation of advanced glycation end product modified proteins. In yet another aspect, the 3DG function inhibitor comprises a structure of an N-methyl-glucamine-like compound, or is arginine or a derivative or modification thereof.

It should be understood that compositions and methods for inhibiting pathways, events, and precursors leading to the synthesis or production of 3DG, may inhibit not only 3DG synthesis, but also its accumulation, and ultimately its function. The invention should be construed to include compositions and methods to inhibit all pathways and precursors leading to 3DG synthesis (see Figures 1 and 2).
In another embodiment of the invention, the disclosure provides methods for directly inhibiting function of 3DG which is associated with various skin diseases and disorders. In one aspect, the method of inhibiting 3DG function in skin includes inhibiting 3DG with compounds such as those comprising structural formulas similar to N-methy-glucamine-like compounds as described herein. Compounds comprising these formulas can bind to 3DG and/or inhibits its function, as described herein. In addition, the invention includes other molecules which can bind to and block 3DG function.

It should be understood that the compounds described herein are not the only compounds capable of inhibiting 3DG function or of treating a 3DG associated skin disease or disorder or diseases and disorders of other tissues and cells. It will be recognized by one of skill in the art that the various embodiments of the invention as described herein related to inhibition of 3DG function, also encompass other methods and compounds useful for inhibiting 3DG function. It will also be recognized by one of skill in the art that other compounds and techniques can be used to practice the invention. The invention should be construed to include compounds and methods useful not merely for their ability to inhibit 3DG function and to treat a 3DG associated skin disease or disorder, but should be construed to also include the ability to inhibit the function of other members of the alpha-dicarbonyl sugar family of compounds, including glyoxal, methyl glyoxal and glucosone. The invention should also be construed to include treating 3DG associated diseases and disorders other than those of skin, such as 3DG associated diseases and disorders of the gums.

In another embodiment, the invention provides multi-component compositions for the inhibition of 3DG and 3DG function. It will be understood by the skilled artisan, in view of the disclosure set forth herein, that certain active components, excipients, additives, adjuvants, and the like, may be added to a composition in order to enhance or otherwise modulate the activity of a compound that inhibits 3DG and/or 3DG function. In one aspect, the invention includes a composition comprising cocoa butter, shea butter, aloe oil, vitamin E, glycerol, water, dimethicone and Natipide II, along with arginine-HCl and meglumine-HCl. As will be understood by the skilled artisan, based on the present disclosure, the ratios and concentrations of the individual components of a composition set forth herein can be adjusted in order to modulate the activity of the composition with respect to 3DG.
That is, the assays and methods provided herein can be used to determine the effect of the individual components in a composition based on the disclosure set forth herein.

**Assays for Testing Inhibition of 3DG and Other Alpha-dicarbonyl**

Sugar Synthesis., Formation, Accumulation, and Function

The present disclosure provides a series of assays for identifying inhibitors of 3DG synthesis, formation, accumulation, and function, as well as measuring the effects of the various inhibitors on 3DG synthesis, formation, accumulation, and function. The assays also include those used to measure 3DG degradation, detoxification, and clearance. The assays of the invention include, but are not limited to, HPLC assays, electrophoretic assays, gas chromatographic-mass spectroscopic assays, amino acid analysis, enzyme activity assays, advanced glycation assays, protein crosslinking assays, NMR analysis, ion exchange chromatography, various chemical analyses, various labeling techniques, surgical and gross dissection techniques, KNA isolation, RT-PCR, histologic techniques, various chemical, biochemical, and molecular synthesis techniques, teratogenicity, mutagenicity, and carcinogenicity assays, urine assays, excretion assays, and a variety of animal, tissue, blood, plasma, cell, biochemical, and molecular techniques. Synthetic techniques may be used to produce compounds, such as: chemical and enzymatic production of FL3P (Examples 1, 2 and 3); polyolysine (Example 4); 3-O-methylsorbitol lysine (Example 8); fructosyl spermine (Example 9); and glycated protein diet (Example 13). Other techniques may be used which are not described herein, but are known to those of skill in the art.

In one embodiment of the invention, standards may be used when testing new agents or compounds or when measuring the various parameters described herein. For example, fructose-lysine is a known modulator of 3DG and 3DF and it can be administered to a group or subject as a standard or control against which the effects of a test agent or compound can be compared. In addition, when measuring a parameter, measurement of a standard can include measuring parameters such as 3DG or 3DF concentrations in a tissue or fluid obtained from a subject before the subject is treated with a test compound and the same parameters can be measured after treatment with the test compound. In another aspect of the invention, a standard can be an exogenously added standard which is an agent or compound that is added to
a sample and is useful as an internal control, especially where a sample is processed through several steps or procedures and the amount of recovery of a marker of interest at each step must be determined. Such exogenously added internal standards are often added in a labeled form, i.e., a radioactive isotope.

Methods for Diagnosing 3DG Associated Skin Diseases or Disorders

The present invention discloses the presence of 3DG in skin and methods for measuring 3DG levels in the skin and for measuring an enzyme responsible for 3DG synthesis in the skin (see Examples 19 and 20). The invention also encompasses methods which may be used to diagnose changes in 3DG levels in the skin which may be associated with wrinkling, aging, or various other skin diseases or disorders. The invention should not be construed to include only methods for diagnosing 3DG associated skin diseases and disorders, but should be construed to include methods for diagnosing skin diseases and disorders associated with other alpha-dicarbonyl sugars as well. The invention should also be construed to include methods for diagnosing 3DG associated diseases or disorders of other cells and tissues as well, including, but not limited to, gum diseases and disorders.

In one embodiment of the invention, a patient with skin wrinkling, skin aging, or another skin disease or disorder, may be subjected to a diagnostic test to determine, for example, the levels of 3DG, the functional activity of 3DG, the levels of 3DF, a 3DF/3DG ratio, the amount of amadorase protein or mRNA present, or the levels of amadorase activity in their skin. Such a test is based on the various methods and assays described herein, or known to those of skill in the art. A higher level of 3DG or amadorase, or their activities, or lower levels of 3DF, compared to a non-affected area of skin or to skin of a normal patient, would be an indication that the skin wrinkling, skin aging, or other skin disease or disorder, is associated with 3DG and that a 3DG inhibitor of the present invention would be an appropriate treatment for the problem. The invention should also be construed to include skin diseases and disorders associated with molecules of the alpha-dicarbonyl sugar family other than 3DG.

In one aspect of the invention, additional markers of 3DG associated skin diseases or disorders can be measured, including, but not limited to, measuring
3DF and FL levels, crosslinked protein levels, as well as levels of other alpha-dicarbonyl sugars such as glyoxal, methyl glyoxal, and glucosone.

A multitude of assays for measuring 3DG levels and function, including measuring its precursors, are described throughout the present disclosure (see Examples 1-22). However, the invention should not be construed to include only the assays described herein, but should be construed to include other assays to measure 3DG levels or function, including assays or techniques which are indirect measures of 3DG levels or functional activity. For example, in one aspect of the invention, indirect measurement of 3DG levels and function can be determined by measuring such things as levels of 3DF, protein crosslinking, proteoglycan crosslinking, or any other assay shown to be correlative of 3DG levels.

In one aspect of the invention, the sample to be used for measuring 3DG levels, etc., is a skin sample. Skin samples may be obtained by methods which include, but are not limited to, punch biopsies, scraping, and blistering techniques.

In another aspect of the invention, indirect assays for 3DG levels or function in the skin which are correlative of 3DG associated skin diseases or disorders may be used. The assays may include, but are not limited to, assays for measuring 3DG levels or function in other tissues, sweat, blood, plasma, saliva, or urine.

The invention discloses a method for diagnosing a 3DG or other alpha-dicarbonyl sugar associated skin disease or disorder comprising acquiring a biological sample from a test subject and comparing the level of 3DG or other alpha-dicarbonyl sugar associated parameter of wrinkling, aging, disease, or disorder of the skin with the level of the same parameter in an otherwise identical biological sample from a control subject. The control can be from an unaffected area of the same subject or from a subject not affected by a 3DG or other alpha-dicarbonyl sugar associated skin disease or disorder. A higher level of the parameter in the test subject is an indication that the test subject has a 3DG or other alpha-dicarbonyl sugar associated wrinkling, aging, disease, or disorder of the skin. The parameters which can be measured are described herein or are known to those of skill in the art, and include, but are not limited to, 3DG, protein crosslinking, proteoglycan crosslinking, advanced glycation end product modified proteins, 3DF, fructosamine kinase/amadorase levels and activity, and fructosamine kinase/amadorase mRNA a changes in levels of reactive oxygen species.
In yet another aspect of the invention, 3DG or other alpha-dicarbonyl sugars may be associated with skin diseases, disorders and conditions and the appearance of these diseases, disorders and conditions selected from the group comprising skin aging, photoaging, skin wrinkling, skin cancer, hyperkeratosis, hyperplasia, acanthosis, papillomatosis, dermatosis, hyperpigmentation, rhinophyma, scleroderma, rosacea, and telangiectasia. In another aspect of the invention, 3DG is associated with functions including, but not limited to, protein crosslinking, mutagenicity, teratogenicity, apoptosis, oxidative damage caused by formation of reactive oxygen species, and cytotoxicity. It is understood that 3DG and other alpha-dicarbonyl sugars are associated with functions causing damage to not only proteins, but to lipids and DNA as well. In aspect of the invention, 3DG or other alpha-dicarbonyl sugars may also be associated with diseases and disorders of the skin (including, but not limited to the mucosa), including, but not limited to, gum diseases and disorders, vaginal and anal mucosa diseases, and the like.

In yet another aspect of the invention, the assays for measuring 3DG levels and function may be used in conjunction with other methods for measuring skin diseases and disorders, such as measuring the thickness or elasticity and/or moisture of the skin. Many of these assays are described herein. One of skill in the art will appreciate that other assays not described herein may be used in conjunction with the 3DG assays to form a complete diagnosis of the type of skin problem involved and whether or not it is a 3DG associated skin problem.

The invention should not be construed to include diagnoising a skin disease, condition or disorder merely by measuring levels of the alpha-dicarbonyl sugar 3DG, it should also be construed to include measuring levels of other members of the alpha-dicarbonyl sugar family as well, as well as their breakdown products, including, but not limited to, 3-deoxyfructose.

Thus, the use of a diagnostic assay to determine an association between 3DG and a skin disease or disorder will allow the selection of appropriate subjects before initiating treatment with an inhibitor of 3DG.

**Methods for Inhibiting or Treating 3DG or Other Alpha-dicarbonyl Sugar Associated Skin Wrinkling, Skin Aging, or Other Skin Disease, Disorder or Condition**
The invention also discloses methods for inhibiting or treating 3DG related skin diseases or disorders. Some examples of 3DG associated diseases or disorders include, but are not limited to, skin cancer, psoriasis, aging, wrinkling, hyperkeratosis, hyperplasia, acanthosis, papillomatosis, dermatosis, rhinophyma, telangiectasia, and rosacea. A cancer or other disease or disorder may belong to any of a group of cancers or other diseases or disorders, which have been described herein, as well as any other related cancer or other disease or disorder known to those of skill in the art.

The invention should not be construed as being limited solely to these examples, as other 3DG associated diseases or disorders which are at present unknown, once known, may also be treatable using the methods of the invention. One of skill in the art would appreciate that 3DG inhibitors may be used prophylactically for some diseases or disorders of the skin, wherein 3DG is known, or it becomes known, that 3DG is associated with a skin disease or disorder. For example, 3DG inhibitors may be applied to prevent wrinkling or other skin problems in subjects who are exposed to harsh environmental elements such as the sun (photoaging/photodamage), heat, chemicals, or cold. Such problems can be due to damage to proteins or other molecules such as lipids or nucleic acids caused by 3DG or alpha-dicarbonyl sugars.

One skilled in the art would appreciate, based upon the disclosure provided herein, that the present invention encompasses methods for prevention of the loss of microcirculation and/or neuro-innervation in the aging, sclerodermic and/or diabetic skin since 3DG increases oxidative stress and AGEs and they, in turn, are linked to neuropathy and circulatory dysfunction.

The present invention also encompasses methods for prevention of hair loss associated with or mediated by loss of microcirculation and/or loss of neuro-innervation in populations of aging, sclerodermic and/or in diabetic individuals. This is because 3DG is a known precursor to the formation of AGEs which are known to be causally connected to the development of neuropathy. Preliminary data demonstrated that diabetic rats treated with DYN 12 and measured for muscle strength while alert had stronger muscle strength than diabetic rats not so treated. This supports the concept that maintenance of nerve conduction and microcirculation that supports nerve innervation is deleteriously affected not only by AGEs, but also
3DG. Similarly, where 3DG would cause blockage of the microcirculation that supports nerve innervation of the hair follicle, the hair follicle will atrophy and die, as is the case in neuropathy. Accordingly, the present invention includes methods for preventing hair loss, where such hair loss is associated with or mediated by the presence of 3DG in the skin proximal to a hair follicle/shaft.

Similarly, the invention includes methods for prevention of graying of hair. This is because, as discussed previously with regard to hair loss, inhibiting the presence and/or activity of 3DG in skin associated with a hair follicle or shaft can prevent the deleterious effect of 3DG on microcirculation affecting such hair and, in turn, preventing the graying of the hair due to such deleterious effect.

Thus, one skilled in the art would appreciate, based upon the disclosure provided herein, that the present invention encompasses methods and compositions relating to prevention of hair loss and/or hair graying. Such compositions and methods encompass, but are not limited to, shampoo or other composition that can be applied to hair and skin associated with a hair follicle to administer the compounds of the invention such that formation, accumulation and/or function of 3DG and/or amadorase is inhibited thereby. Based on the disclosure provided herein, the skilled artisan would understand that such compounds include, but are not limited to, meglumine. Further, the formulation of compositions to be applied to hair follicles and the dosage and treatment regimens therefore, are disclosed herein and are also well-known to those in the art.

The invention encompasses methods for treatment of skin wound healing. This is because ROS are associated with the origination of wounds. Accordingly, the skilled artisan would appreciate, based upon the disclosure provided herein, that any inhibitor of ROS will positively effect wound healing. Given 3DG’s role in the originatin of ROS, inhibiting ROS by inhibiting the productin of 3DG can result in methods useful to prevent and treat wounds. Further support for use of 3DG inhibition in skin as a useful wound healing therapeutic is provided by studies demonstrating that diabetics are especially prone to wound healing problems, since as previously discussed elsewhere herein, diabetics have elevated levels of 3DG and detoxify the 3DG less efficiently than non diabetics. Thus, the surprising finding that 3DG, as well as the enzyme responsible for its enzymatic synthesis, are present in
skin makes possible, for the first time, the development of novel therapeutics for
promotion of wound healing, especially for diabetics.

Since 3DG and the pathway for its formation, are present in skin, and
are involved in the production of ROS and since ROS are, in turn, involved in
inflammation, the skilled artisan would also appreciate that the invention
encompasses methods for treating or ameliorating diseases, disorders or conditions
associated with mucosal inflammation. Inhibition of 3DG formation, function,
and/or accumulation in skin can inhibit mucosal inflammation such that conditions
associated with inflammation of the mucosa (e.g., nasal passages, vagina, rectum,
mouth cavity, and the like) can be inhibited by such inhibition. For instance,
inhibition of 3DG can be used to modulate browning of teeth, inflammation of the
mouth, gingivitis, periodontal disease, herpes sores, and the like.

Further, because inhibiting 3DG can prevent mucosal inflammation
and can induce wound healing, such inhibition can also provide a useful therapeutics
for the prevention and treatment of viral, bacterial or fungal infection where the
infection is mediated by pathogenic infection via the skin and/or mucosa. Therefore,
the present invention includes methods and compositions for prevention or treatment
of fungal, viral and bacterial infection by providing an inactivator of amadorase
and/or 3DG to a patient in need of such treatment.

The invention encompasses methods of treating or preventing
gingivitis, periodontal diseases, yellowing of the teeth, and the like. This is because
the data disclosed herein demonstrate that 3DG is present in saliva, and is present in
skin, indicating that it is present in mucosa. Thus, one skilled in the art would
appreciated, based upon the disclosure provided herein, that inhibition of 3DG
associated with the mucosa in the mouth cavity can inhibit the deleterious effects
associated with or mediated by the molecule, including, but not limited to, gingivitis,
periodontal disease, and discoloration of the teeth. This is because oxidative stress
and AGEs are associated with these conditions and 3DG induces oxidative stress and
AGEs. Further, the skilled artisan, armed with the teachings provided herein, would
understand that the present invention encompasses methods of treating Wilson's
disease, rheumatoid arthritis, progressive systemic sclerosis, fibrotic lung disease,
Raynaud's phenomenon, joint contractures, Sjogren's syndrome, and the like. This is
because, 3DG causes the induction of reactive oxygen species and reactive oxygen
species cause inflammation, diseases associated with inflammation mediated by or associated with ROS can be prevented or treated by inhibition of 3DG. Therefore Wilson's disease, rheumatoid arthritis, progressive systemic sclerosis, fibrotic lung disease, Raynaud's phenomenon, joint contractures, Sjogren's syndrome, and the like, can be treated according to the methods set forth herein relating to inhibiting 3DG and/or amadorase.

The present invention includes methods of treating breast cancer. This is because, as more fully set forth elsewhere herein, the data disclosed herein demonstrate that 3DG is present in sweat. Because mammary glands are highly specialized sweat glands, the skilled artisan would appreciate, based upon the disclosure provided herein, that inhibition of 3DG in such tissue would provide a beneficial effect given the deleterious effects associated with or mediated by 3DG.

Inhibiting 3DG in skin, as appreciated by the skilled artisan based upon the disclosure provided herein, can provide useful therapeutics for treatment of breast cancer because 3DG causes oxidative stress and the formation of reactive oxygen and inhibits enzymes that combat oxidative stress. Thus, 3DG depletes the body's defenses against inflammation, in particular, high levels of 3DG present in skin deleteriously depletes the defenses present in the skin and mucosa. Thus, without wishing to be bound by any particular theory, the effects of 3DG are primarily due to its effect on oxidative stress and, in turn, to the entire inflammatory cascade. That is important for breast cancer where it is believed that long term oxidative stress, and not a single point mutation, causes the disease.

Likewise, one of skill in the art, once armed with the teachings disclosed herein, would understand that where a bodily fluid, such as saliva, sweat, lymph, urine, semen, and blood, comprising 3DG, is produced by or associated with skin, a disease, disorder or condition mediated by the contact of such fluid with a cell, tissue or organ can be treated by inhibition of 3DG. Such disease, disorder or condition mediated by or associated with 3DG present in a bodily fluid includes, but is not limited to, non-Hodgkins Lymphoma, where sweat comprising 3DG saturates the lymph glands.

Further, the invention includes methods of inhibiting formation of 3DG adducts, and/or inactivating these adducts, since these adducts will also contribute to diseases, disorders or conditions associated with 3DG, including those disclosed.
elsewhere herein. That is, like prevention of formation, accumulation, and/or functioning of 3DG prevents the deleterious effects of the compound relating to aging and disease, and more specifically, to the deleterious effects of 3DG on skin as disclosed elsewhere herein, inhibiting the deleterious effects of 3DG adducts and/or intermediates wherever found will likewise prevent their deleterious effects. The skilled artisan, once armed with the teachings provided herein, would understand that such 3DG adducts/intermediates include, but are not limited to, those depicted in Figure 18, and that such intermediates/adducts that form from 3DG that will also contribute to aging and disease, wherever found.

These adducts are heretofore unknown, and the skilled artisan would appreciate, based on their novel disclosure herein, that inhibiting such adducts will inhibit a disease process mediated by or associated therewith, in skin and wherever such adducts are present. Thus, the present invention encompasses inhibiting the synthesis, formation and accumulation of such 3DG adducts, wherever they are detected using detection methods disclosed herein, known in the art, or to be developed in the future.

The present invention encompasses methods for treating or ameliorating a wide plethora of diseases, which diseases are mediated by or associated with changes in skin due to the interactions of 3DG with proteins in skin, such as, e.g., collagen and elastin, and with the induction of ROS and their subsequent reaction with components of skin. That is, the data disclosed herein demonstrate that 3DG in the skin mediates or is associated with collagen cross-linking and, in turn, with skin thickening, such that preventing the accumulation, formation, function, and/or increasing the clearance of 3DG and/or Amadorase, from the skin can provide a therapeutic benefit for a disease disorder or condition mediated by or associated with such thickening.

In addition, the present invention encompasses treating or ameliorating a disease, disorder or condition mediated by or associated with, oxidative stress. This is because 3DG induces oxidative stress. i.e., 3DG induces oxidative stress either directly or through the formation of AGEs and therefore 3DG is involved in the inflammatory response. Thus, inhibiting 3DG will treat or prevent a disease, disorder or condition associated with inflammation. Such disease, disorder or condition includes, but is not limited to, gingivitis, periodontal disease, browning/yellowing of
teeth, herpes lesions, and scarring since these are mediated by, or associated with, ROS. Accordingly, preventing ROS, such as by, for instance, treatment of the teeth and/or oral tissue (e.g., gums, and the like) with an inhibitor of 3DG, e.g., meglumine, can reduce deleterious effects of ROS in the buccal cavity such as the aforementioned diseases, disorders or conditions.

The present invention further encompasses treatments that affect the appearance of skin based upon inhibition of 3DG, its adducts/intermediates, as well as inhibition of amadorase and the synthesis of 3DG. Thus, even where the condition, disorder or disease is not treated or ameliorated, the invention includes methods of treatment that affect the appearance of the skin such that, at the very least, the condition, disorder or disease affects the appearance of the skin to a lesser degree than the in the absence of the treatment. These treatments are therefore cosmetic and can produce an improvement in physical appearance.

The present invention includes methods of treating skin aging related to the loss of skin elasticity. This is because, as more fully set forth elsewhere herein, the data disclosed herein demonstrate, for the first time, that 3DG and the enzyme associated with its synthesis, are present in skin and that inhibition of 3DG can prevent or reverse the loss of skin elasticity associated with its presence in skin. Accordingly, the skilled artisan would appreciate, once armed with the teachings provided herein, that inhibiting 3DG in skin can reduce skin aging such that the present invention provides useful therapeutics for inhibiting skin aging and loss of skin elasticity. The skilled artisan would further understand that skin aging therapeutics encompass, but are not limited, to various treatment procedures well-known in the dermatological and cosmetological arts including, but not limited to, skin wraps, exfoliants, masks, and the like, that can be used to effectuate the various treatments disclosed herein.

The invention encompasses methods of preventing the susceptibility to viral, fungal and bacterial infections especially in oral, rectal and vaginal routes by inhibiting Amadorase and/or by inactivating 3DG. Specifically, susceptibility to infection by, e.g., HIV, papillomavirus and Epstein-Barr virus can be decreased because changes in skin affect receptivity to disease and 3DG induces the formation of ROS and AGEs and also actively interacts with skin proteins, in particular collagen and elastin, therefore they affect the skin such that receptivity is altered.
One skilled in the art would understand, based upon the disclosure provided herein, that the present invention provides useful therapeutics for a wide plethora of diseases, disorders or conditions associated with 3DG in skin. This is because, *inter alia*, it is well-known in the art that 3DG mediates formation of ROS, which, in turn, are well-known to be involved in a wide variety of diseases, disorders or conditions as set forth herein.

The invention also includes methods for inhibiting or treating skin diseases or disorders associated with members of the alpha-dicarbonyl sugar family of compounds other than 3DG.

In one aspect of the invention, various changes in the skin can be measured following treatment with inhibitors of 3DG. The skin topography can be defined by parameters such as: (a) number of wrinkles; (b) total area of wrinkles; (c) total length of wrinkles; (d) mean length of wrinkles; and (e) mean depth of wrinkles. The type of wrinkles can be determined on the basis of depth, length, and area. These properties can be used when evaluating the changes in skin due to disease or disorder or the effects of a treatment on the skin. The effects of changes in 3DG levels and function on various skin qualities can be determined based on techniques known in the art. Methods to measure skin quality include, but are not limited to, measuring viscoelastic properties with instruments such as a ballistometer, measuring the mechanical/vertical deformation properties of the skin with an instrument such as a cutometer, or measuring changes in skin capacitance resulting from changes in the degree of hydration using a corneometer.

**Compositions and Methods for Administration**

The invention relates to the administration of an identified compound in a pharmaceutical or cosmetic composition to practice the methods of the invention, the composition comprising the compound or an appropriate derivative or fragment of the compound and a pharmaceutically-acceptable carrier. For example, a chemical composition with which an appropriate inhibitor of enzyme dependent or nonenzyme dependent production of 3DG, or inhibitor of 3DG accumulation or function, or stimulator of 3DG removal, detoxification, or degradation, is combined, is used to administer the appropriate compound to an animal. The invention should be construed to include the use of one, or simultaneous use of more than one, inhibitor of
3DG or stimulator of 3DG removal, degradation, or detoxification. When more than one stimulator or inhibitor is used, they can be administered together or they can be administered separately.

In one embodiment, the pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 mg/kg/day. In another embodiment, the pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 g/kg/day.

In another embodiment of the invention, a pharmaceutical composition is in the form of a liposome crème. In one aspect, a composition comprises 23.9 grams of BIOCREME Concentrate (BioChemica International Inc.), blended with 2.9 grams cocoa butter, 1.4 grams shea butter, 2.2 grams aloe oil, 1.1 grams vitamin E, 3.7 grams glycerol, 51 grams water, 1.1 grams dimethicone and 10.8 grams Natipide II, along with 1 gram arginine-HCl and 1 gram meglumine-HCl. However, the invention should not be limited to a liposome-based delivery vehicle.

In another embodiment, a composition of the invention may omit Arginine from the liposome crème formulation set forth above. In yet another embodiment, a composition of the invention may substitute any one of the compounds set forth in Table H for meglumine in the liposome crème formulation set forth above. However, a composition of the invention should not be limited to include these compounds, but rather, should be construed to include any compound as described herein as being useful in the present invention.

As will be understood by the skilled artisan, when armed with the disclosure set forth herein, a composition useful in the present invention can include one active ingredient. Alternatively, a composition useful in the present invention can include at least two active ingredients. In one aspect, multiple active ingredients may be active in a additive manner. In another aspect, multiple active ingredients may be active in a synergistic manner. That is, the multiple active ingredients in a composition of the invention may provide a therapeutic effect that is greater than the addition of the therapeutic effects provided by each of the active ingredients alone.

By way of a non-limiting example, a composition can comprise both an inhibitor of alpha-dicarbonyl sugar formation and an inhibitor of alpha-dicarbonyl sugar function or effect, together exhibit a synergistic effect in the alleviation of alpha-dicarbonyl
sugar-associated conditions, as compared with compositions comprising either type of inhibitor alone. In one embodiment, the combination of meglumine and arginine for the treatment of alpha-dicarbonyl sugar-associated conditions.

Other pharmaceutically acceptable carriers which are useful include, but are not limited to, glycerol, water, saline, ethanol and other pharmaceutically acceptable salt solutions such as phosphates and salts of organic acids. Examples of these and other pharmaceutically acceptable carriers are described in Remington's Pharmaceutical Sciences (1991, Mack Publication Co., New Jersey).

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butanediol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides.

Pharmaceutical compositions that are useful in the methods of the invention may be administered, prepared, packaged, and/or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

The compositions of the invention may be administered via numerous routes, including, but not limited to, oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, or ophthalmic administration routes. The route(s) of administration will be readily apparent to the skilled artisan and will depend upon any number of factors including the type and severity of the disease being treated, the type and age of the veterinary or human patient being treated, and the like.

Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically in oral solid formulations, ophthalmic, suppository, aerosol, topical or other similar formulations. In addition to the compound such as heparan sulfate, or a biological equivalent thereof, such
pharmaceutical compositions may contain pharmaceutically-acceptable carriers and other ingredients known to enhance and facilitate drug administration. Other possible formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer compounds according to the methods of the invention.

Compounds which are identified using any of the methods described herein may be formulated and administered to a mammal for treatment of skin aging, skin wrinkling, and various skin related diseases, disorders, or conditions described herein.

The invention encompasses the preparation and use of pharmaceutical compositions comprising a compound useful for treatment of various skin related diseases, disorders, or conditions described herein, including skin aging, photoaging, and wrinkling of the skin. The invention also encompasses 3DG associated diseases and disorders other than those of the skin, including, but not limited to, gum diseases and disorders. Such a pharmaceutical composition may consist of the active ingredient alone, in a form suitable for administration to a subject, or the pharmaceutical composition may comprise at least one active ingredient and one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The active ingredient may be present in the pharmaceutical composition in the form of a physiologically acceptable ester or salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

An obstacle for topical administration of pharmaceuticals is the stratum corneum layer of the epidermis. The stratum corneum is a highly resistant layer comprised of protein, cholesterol, sphingolipids, free fatty acids and various other lipids, and includes cornified and living cells. One of the factors that limits the penetration rate (flux) of a compound through the stratum corneum is the amount of the active substance which can be loaded or applied onto the skin surface. The greater the amount of active substance which is applied per unit of area of the skin, the greater the concentration gradient between the skin surface and the lower layers of the skin, and in turn the greater the diffusion force of the active substance through the skin. Therefore, a formulation containing a greater concentration of the active substance is more likely to result in penetration of the active substance through the
skin, and more of it, and at a more consistent rate, than a formulation having a lesser concentration, all other things being equal.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts.

Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs.

Pharmaceutical compositions that are useful in the methods of the invention may be prepared, packaged, or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, intrathecal or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.
The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active agents. Particularly contemplated additional agents include anti-emetics and scavengers such as cyanide and cyanate scavengers.

Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.

Formulations suitable for topical administration include, but are not limited to, liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically-administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

Enhancers of permeation may be used. These materials increase the rate of penetration of drugs across the skin. Typical enhancers in the art include ethanol, glycerol monolaurate, PGML (polyethylene glycol monolaurate), dimethylsulfoxide, and the like. Other enhancers include oleic acid, oleyl alcohol, ethoxydiglycol, laurocapram, alkanecarboxylic acids, dimethylsulfoxide, polar lipids, or N-methyl-2-pyrrolidone.

One acceptable vehicle for topical delivery of some of the compositions of the invention may contain liposomes. The composition of the liposomes and their use are known in the art (for example, see Constanza, U.S. Patent No. 6,323,219).

The source of active compound to be formulated will generally depend upon the particular form of the compound. Small organic molecules and peptidyl or oligo fragments can be chemically synthesized and provided in a pure form suitable
for pharmaceutical/cosmetic usage. Products of natural extracts can be purified according to techniques known in the art. Recombinant sources of compounds are also available to those of ordinary skill in the art.

In alternative embodiments, the topically active pharmaceutical or cosmetic composition may be optionally combined with other ingredients such as moisturizers, cosmetic adjuvants, anti-oxidants, chelating agents, bleaching agents, tyrosinase inhibitors and other known depigmentation agents, surfactants, foaming agents, conditioners, humectants, wetting agents, emulsifying agents, fragrances, viscosifiers, buffering agents, preservatives, sunscreens and the like. In another embodiment, a permeation or penetration enhancer is included in the composition and is effective in improving the percutaneous penetration of the active ingredient into and through the stratum corneum with respect to a composition lacking the permeation enhancer. Various permeation enhancers, including oleic acid, oleyl alcohol, ethoxydiglycol, laurocapram, alkanecarboxylic acids, dimethylsulfoxide, polar lipids, or N-methyl-2-pyrrolidone, are known to those of skill in the art. In another aspect, the composition may further comprise a hydrotropic agent, which functions to increase disorder in the structure of the stratum corneum, and thus allows increased transport across the stratum corneum. Various hydrotropic agents such as isopropyl alcohol, propylene glycol, or sodium xylene sulfonate, are known to those of skill in the art. The compositions of this invention may also contain active amounts of retinoids (i.e., compounds that bind to any members of the family of retinoid receptors), including, for example, tretinoin, retinol, esters of tretinoin and/or retinol and the like.

The topically active pharmaceutical or cosmetic composition should be applied in an amount effective to affect desired changes. As used herein "amount effective" shall mean an amount sufficient to cover the region of skin surface where a change is desired. An active compound should be present in the amount of from about 0.0001% to about 15% by weight volume of the composition. More preferable, it should be present in an amount from about 0.0005% to about 5% of the composition; most preferably, it should be present in an amount of from about 0.001% to about 1% of the composition. Such compounds may be synthetically-or naturally-derived.
Liquid derivatives and natural extracts made directly from biological sources may be employed in the compositions of this invention in a concentration (w/v) from about 1 to about 99%. Fractions of natural extracts and protease inhibitors may have a different preferred range, from about 0.01% to about 20% and, more preferably, from about 1% to about 10% of the composition. Of course, mixtures of the active agents of this invention may be combined and used together in the same formulation, or in serial applications of different formulations.

The composition of the invention may comprise a preservative from about 0.005% to 2.0% by total weight of the composition. The preservative is used to prevent spoilage in the case of an aqueous gel because of repeated patient use when it is exposed to contaminants in the environment from, for example, exposure to air or the patient's skin, including contact with the fingers used for applying a composition of the invention such as a therapeutic gel or cream. Examples of preservatives useful in accordance with the invention included but are not limited to those selected from the group consisting of benzyl alcohol, sorbic acid, parabens, imidurea and combinations thereof. A particularly preferred preservative is a combination of about 0.5% to 2.0% benzyl alcohol and 0.05% to 0.5% sorbic acid.

The composition preferably includes an antioxidant and a chelating agent which inhibit the degradation of the compound for use in the invention in the aqueous gel formulation. Preferred antioxidants for some compounds are BHT, BHA, alphatocopherol and ascorbic acid in the preferred range of about 0.01% to 0.3% and more preferably BHT in the range of 0.03% to 0.1% by weight by total weight of the composition. Preferably, the chelating agent is present in an amount of from 0.01% to 0.5% by weight by total weight of the composition. Particularly preferred chelating agents include edetate salts (e.g. disodium edetate) and citric acid in the weight range of about 0.01% to 0.20% and more preferably in the range of 0.02% to 0.10% by weight by total weight of the composition. The chelating agent is useful for chelating metal ions in the composition which may be detrimental to the shelf life of the formulation. While BHT and disodium edetate are the particularly preferred antioxidant and chelating agent respectively for some compounds, other suitable and equivalent antioxidants and chelating agents may be substituted therefor as would be known to those skilled in the art.
Controlled-release preparations may also be used and the methods for the use of such preparations are known to those of skill in the art.

In some cases, the dosage forms to be used can be provided as slow or controlled-release of one or more active ingredients therein using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, or microspheres or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the pharmaceutical compositions of the invention. Thus, single unit dosage forms suitable for oral administration, such as tablets, capsules, gelcaps, and caplets, that are adapted for controlled-release are encompassed by the present invention.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts.

Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood level of the drug, and thus can affect the occurrence of side effects.

Most controlled-release formulations are designed to initially release an amount of drug that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body.

Controlled-release of an active ingredient can be stimulated by various inducers, for example pH, temperature, enzymes, water, or other physiological conditions or compounds. The term "controlled-release component" in the context of the present invention is defined herein as a compound or compounds, including, but not limited to, polymers, polymer matrices, gels, permeable membranes, liposomes, or...
microspheres or a combination thereof that facilitates the controlled-release of the active ingredient.

Liquid suspensions may be prepared using conventional methods to achieve suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include, for example, water, and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions may further comprise one or more additional ingredients including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose. Known dispersing or wetting agents include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene steaate, heptadecaethyleneoxyacetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin, and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl-para-hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water, and isotonic
saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

As used herein, an "oily" liquid is one which comprises a carbon-containing liquid molecule and which exhibits a less polar character than water.

A formulation of a pharmaceutical composition of the invention suitable for oral administration may be prepared, packaged, or sold in the form of a discrete solid dose unit including, but not limited to, a tablet, a hard or soft capsule, a cachet, a troche, or a lozenge, each containing a predetermined amount of the active ingredient. Other formulations suitable for oral administration include, but are not limited to, a powdered or granular formulation, an aqueous or oily suspension, an aqueous or oily solution, a paste, a gel, a toothpaste, a mouthwash, a coating, an oral rinse, or an emulsion. The terms oral rinse and mouthwash are used interchangeably herein.
A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for oral or buccal administration. Such a formulation may comprise, but is not limited to, a gel, a liquid, a suspension, a paste, a toothpaste, a mouthwash or oral rinse, and a coating. For example, an oral rinse of the invention may comprise a compound of the invention at about 1.4%, chlorhexidine gluconate (0.12%), ethanol (11.2%), sodium saccharin (0.15%), FD&C Blue No. 1 (0.001%), peppermint oil (0.5%), glycerine (10.0%), Tween 60 (0.3%), and water to 100%. In another embodiment, a toothpaste of the invention may comprise a compound of the invention at about 5.5%, sorbitol, 70% in water (25.0%), sodium saccharin (0.15%), sodium lauryl sulfate (1.75%), carbopol 934, 6% dispersion in (15%), oil of spearmint (1.0%), sodium hydroxide, 50% in water (0.76%), dibasic calcium phosphate dihydrate (45%), and water to 100%. The examples of formulations described herein are not exhaustive and it is understood that the invention includes additional modifications of these and other formulations not described herein, but which are known to those of skill in the art.

A tablet comprising the active ingredient may, for example, be made by compressing or molding the active ingredient, optionally with one or more additional ingredients. Compressed tablets may be prepared by compressing, in a suitable device, the active ingredient in a free-flowing form such as a powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, an excipient, a surface active agent, and a dispersing agent. Molded tablets may be made by molding, in a suitable device, a mixture of the active ingredient, a pharmaceutically acceptable carrier, and at least sufficient liquid to moisten the mixture. Pharmaceutically acceptable excipients used in the manufacture of tablets include, but are not limited to, inert diluents, granulating and disintegrating agents, binding agents, and lubricating agents. Known dispersing agents include, but are not limited to, potato starch and sodium starch glycollate. Known surface-active agents include, but are not limited to, sodium lauryl sulphate. Known diluents include, but are not limited to, calcium carbonate, sodium carbonate, lactose, macrocrystalline cellulose, calcium phosphate, calcium hydrogen phosphate, and sodium phosphate. Known granulating and disintegrating agents include, but are not limited to, corn starch and alginic acid. Known binding agents include, but are not limited to, gelatin, acacia, pre-gelatinized maize starch, polyvinylpyrrolidone, and hydroxypropyl.
methylcellulose. Known lubricating agents include, but are not limited to, magnesium stearate, stearic acid, silica, and talc.

Tablets may be non-coated or they may be coated using known methods to achieve delayed disintegration in the gastrointestinal tract of a subject, thereby providing sustained release and absorption of the active ingredient. By way of example, a material such as glyceryl monostearate or glyceryl distearate may be used to coat tablets. Further by way of example, tablets may be coated using methods described in U.S. Patents numbers 4,256,108; 4,160,452; and 4,265,874 to form osmotically-controlled release tablets. Tablets may further comprise a sweetening agent, a flavoring agent, a coloring agent, a preservative, or some combination of these in order to provide for pharmaceutically elegant and palatable preparation.

Hard capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such hard capsules may comprise the active ingredient, and may further comprise additional ingredients including, for example, an inert solid diluent such as calcium carbonate, calcium phosphate, or kaolin.

Soft gelatin capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such soft capsules may comprise the active ingredient, which may be mixed with water or an oil medium such as peanut oil, liquid paraffin, or olive oil.

Liquid formulations of a pharmaceutical composition of the invention which are suitable for oral administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for rectal administration. Such a composition may be in the form of, for example, a suppository, a retention enema preparation, and a solution for rectal or colonic irrigation.

Suppository formulations may be made by combining the active ingredient with a non-irritating pharmaceutically acceptable excipient which is solid at ordinary room temperature (i.e., about 20°C) and which is liquid at the rectal temperature of the subject (i.e., about 37°C in a healthy human). Suitable pharmaceutically acceptable excipients include, but are not limited to, cocoa butter,
polyethylene glycols, and various glycerides. Suppository formulations may further comprise various additional ingredients including, but not limited to, antioxidants, and preservatives.

Retention enema preparations or solutions for rectal or colonic irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As is well known in the art, enema preparations may be administered using, and may be packaged within, a delivery device adapted to the rectal anatomy of the subject. Enema preparations may further comprise various additional ingredients including, but not limited to, antioxidants, and preservatives.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for vaginal administration. Such a composition may be in the form of, for example, a suppository, an impregnated or coated vaginally-insertable material such as a tampon, a douche preparation, or gel or cream or a solution for vaginal irrigation.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a chemical composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (i.e., such as with a physiologically degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

Douche preparations or solutions for vaginal irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As is well known in the art, douche preparations may be administered using, and may be packaged within, a delivery device adapted to the vaginal anatomy of the subject.

Douche preparations may further comprise various additional ingredients including, but not limited to, antioxidants, antibiotics, antifungal agents, and preservatives. As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the
composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrastemal injection, and kidney dialytic infusion techniques.

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e., powder or granular) form for reconstitution with a suitable vehicle (e.g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer system. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.
A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conventional methods, and may, for example, 0.1 to 20% (w/w) active ingredient, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder or an aerosolized or atomized solution or suspension comprising the active ingredient. Such powdered, aerosolized, or aerosolized formulations, when dispersed, preferably have an average particle or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the additional ingredients described herein.

As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcants; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" which may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro, ed. (1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA), which is incorporated herein by reference.

Typically, dosages of the compound of the invention which may be administered to an animal, preferably a human, will vary depending upon any number of factors, including but not limited to, the type of animal and type of disease state being treated, the age of the animal and the route of administration.

The compound can be administered to an animal as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors,
such as, but not limited to, the type and severity of the disease being treated, the type and age of the animal, etc.

Compounds of the invention may also be given in combination with one or more additional compounds or compositions. Additional compounds and compositions include, but are not limited to, steroids (e.g., topical steroids, including topical steroids of varying class and strength), acne treatments, antacids, probiotic agents, H-2 blockers, and proton pump inhibitors.

Steroids include, but are not limited to, hydrocortisone, clobetasone butyrate, triamcinolone acetonide, fluocinolone acetonide, betamethasone valerate, betamethasone dipropionate, diflucortolone valerate, fluticasone valerate, hydrocortisone 17-butyrate, mometasone furoate, methylprednisolone aceponate, betamethasone dipropionate, and clobetasol propionate.

Probiotic agents include live microorganisms, including Lactobacillus species, Bifidobacterium species and yeasts, among others, that may beneficially affect the host upon use by improving the balance of microflora associated with the host.

H2-receptor antagonists, also known as "H-2 blockers," include compositions for the prevention and relief of heartburn associated with acid indigestion.

Proton pump inhibitors, as used herein, describes compounds that block production of stomach acid, by inhibiting ("shutting down") a system in the stomach known as the proton pump, also known as the "hydrogen-potassium adenosine triphosphate enzyme system".

It will be recognized by one of skill in the art that the various embodiments of the invention as described above relating to methods of inhibiting 3DG or treating 3DG related diseases or conditions, includes other diseases and conditions not described herein.

Kits

The present invention should be construed to include kits for inhibiting or stimulating 3DG, treating 3DG associated skin diseases and disorders, kits for measuring 3DG and 3DG related parameters, and kits for diagnosing 3DG associated
skin diseases and disorders. The invention should be construed to include kits for alpha-dicarbonyl sugars other than 3DG as well.

The invention includes a kit comprising an inhibitor of 3DG or a compound identified in the invention, a standard, and an instructional material which describes administering the inhibitor or a composition comprising the inhibitor or compound to a cell or an animal. This should be construed to include other embodiments of kits that are known to those skilled in the art, such as a kit comprising a standard and a (preferably sterile) solvent suitable for dissolving or suspending the composition of the invention prior to administering the compound to a cell or an animal. Preferably the animal is a mammal. More preferably, the mammal is a human.

The invention also includes a kit comprising a stimulator of 3DG degradation, detoxification, or clearance, or such a stimulatory compound identified in the invention, a standard, and an instructional material which describes administering the stimulator or a composition comprising the stimulator or compound to a cell or an animal. This should be construed to include other embodiments of kits that are known to those skilled in the art, such as a kit comprising a standard and a (preferably sterile) solvent suitable for dissolving or suspending the composition of the invention prior to administering the compound to a cell or an animal.

In accordance with the present invention, as described above or as discussed in the Examples below, there can be employed conventional chemical, cellular, histochemical, biochemical, molecular biology, microbiology and recombinant DNA techniques which are known to those of skill in the art. Such techniques are explained fully in the literature. See for example, Sambrook et al., 1989 Molecular Cloning - a Laboratory Manual, Cold Spring Harbor Press; Glover, (1985) DNA Cloning: a Practical Approach; Gait, (1984) Oligonucleotide Synthesis; Harlow et al., 1988 Antibodies - a Laboratory Manual, Cold Spring Harbor Press; Roe et al., 1996 DNA Isolation and Sequencing: Essential Techniques, John Wiley; and Ausubel et al., 1995 Current Protocols in Molecular Biology, Greene Publishing.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out the
preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EXAMPLES

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only and the invention should in no way be construed as being limited to these Examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Methods

Transdermal Drug Delivery

There are several advantages to delivering compounds, including drugs or other therapeutic agents, into the body through the skin, a process called transdermal drug delivery. Transdermal drug delivery offers an attractive alternative to injections and oral medications. It provides the capacity for multi day therapy with a single application thereby improving patient compliance. Such delivery would extend the activity of drugs having short half-life through the reservoir of drug present in the delivery system and its controlled release characteristics. Transdermal drug delivery avoids gastrointestinal tract difficulties during absorption caused by enzymes or drug interactions with food. Not only that, it avoids first pass i.e. the initial passage of a drug substance through the systemic and portal circulation. However, applications of transdermal drug delivery are limited to only a few drugs as a result of low skin permeability [Prausnitz, M.R. et al. Current status and future potential of transdermal drug delivery. 2004. Nat Rev Drug Discov 3(2): p.1 15-24].

Transdermal transport of solutes is largely controlled by stratum corneum lipid bilayers. Solute transport in stratum corneum lipid bilayers, like in other lipid bilayer systems, is highly anisotropic and size-dependent. Specifically, lipid bilayers exhibit strong structural heterogeneity that results in spatial variations in solute partition and diffusion coefficients. As a result, molecules are believed to diffuse across skin following a tortuous pathway within either the tail-group (for hydrophobic molecules) or head-group (for hydrophilic molecules) regions, in which transport between bilayers can occur at bilayer-bilayer interfaces or other sites of structural disorganization [Marrink, S.J. and Berendsen, H.J. Permeation Process of

A few drugs will penetrate the skin effectively. Nicotine, estrogen, scopolamine, fentanyl, and nitroglycerine are among the few drugs that can be successfully delivered transdermally from patches simply because they are relatively small and potent at small doses of 0.1 mg to 15 mg/day [Kanikkannan, N. et al. Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. 2000. Curr Med Chem 7(6): p.593-608]. Many other drugs can be delivered only when an additional enhancement system is provided to "force" them to pass through the skin. Among several methods of transdermal drug delivery are electroporation, sonophoresis, iontophoresis, permeation enhancers (cyclodextrins), and liposomes.

Compounds of this invention can be administered via topical use of any of these transdermal delivery methods.

Liposomes

Liposomes are microscopic! fluid-filled pouches whose walls are made of layers of phospholipids identical to those that make up the cell membranes. They are well known and their structures and properties have been thoroughly researched. Essentially, they are small uni- or multi-lamellar lipid/water structures with diameters in the micron range. Liposomes can be formed from a variety of natural phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines. They can be formulated to incorporate a wide range of materials as a payload either in the water or in the lipid compartments.

Liposomes are extremely versatile and are variable due to their composition. They can be used to deliver vaccines, proteins (enzymes), nucleotides, plasmids, drugs, or cosmetics to the body. Liposomes can be used as carriers for lipophilic drugs like the anti-tumor and the anti-viral derivatives of AZT [Kamps, J.A. et al. Preparation and characterization of conjugates of (modified) human serum albumin and liposomes: drug carriers with an intrinsic anti-HIV activity. 1996. Biochim Biophys Acta 1278(2): p.183-90]. Insulin can also be delivered via liposomes [Muramatsu, K. et al. The relationship between the rigidity of the liposomal membrane and the absorption of insulin after nasal administration of
Liposomes modified with an enhancer containing insulin in rabbits. 1999. Drug Dev Ind Pharm 25(10): p.1099-105]. For medical uses as drug carriers, the liposomes can also be injected intravenously and when they are modified with lipids, their surfaces become more hydrophilic and hence the circulation time in the bloodstream can be increased significantly. Such so-called "stealth" liposomes are especially being used as carriers for hydrophilic (water soluble) anti cancer drugs like doxorubicin.


Liposomes are also sometimes used in cosmetics because of their moisturizing qualities. It was found that phospholipids combined with water immediately formed a sphere because one end of each molecule is water soluble, while the opposite end is water insoluble.

Sonophoresis

Sonophoresis or phonophoresis has been widely used in sports medicine since the sixties. Controlled studies in humans in vivo have demonstrated absence or mild effects of the technique with the parameters currently used (frequency 1-3 MHz, intensity 1-2 W/cm(2), duration 5-10 mins, continuous or pulse mode).

However, it was demonstrated in 1995 that administration of macromolecules with conserved biological activity was feasible in animals in vivo using low frequency ultrasound. This led to new research into this method of transdermal administration [Machet, L. and Boucaud, A. Phonophoresis: efficiency, mechanisms and skin tolerance. 2002. Int J Pharm 243(1-2): p.1-15].

In this method, a short application of ultrasound is used to permeabilize skin for a prolonged period of time. The enhancement induced by ultrasound is particularly significant at low-frequencies (f<100 kHz). During this period, ultrasonically permeabilized skin may be utilized for drug delivery. In
addition, a sample of interstitial fluid or its components may be extracted through permeabilized skin for diagnostic applications. Detailed studies on drug delivery have been performed using insulin and mannitol as model drugs. Studies on diagnostics were performed using glucose as a model analyte [Mitragotri, S. and Kost, J. Low-frequency sonophoresis: a noninvasive method of drug delivery and diagnostics. 2000. Biotechnol Prog 16(3): p.488-92].

In vitro, in vivo, as well as clinical studies have also demonstrated the successful effect of low-frequency ultrasound on transdermal drug delivery and glucose extraction. Mechanistic insights gained through a number of investigations have also been reviewed [Mitragotri, S. and Kost, J. Low-frequency sonophoresis: a review. 2004. Adv Drug Deliv Rev 56(5): p.589-601].

At the School of Pharmacy, Faculty of Sciences, University of Geneva, a study was done to shed light on the mechanism(s) by which low-frequency ultrasound (20 KHz) enhances the permeability of the skin. The physical effects on the barrier and the transport pathway, in particular, were examined. The amount of lipid removed from the intercellular domains of the stratum corneum following sonophoresis was determined by infrared spectroscopy. Transport of the fluorescent probes nile red and calcein, under the influence of ultrasound, was evaluated by laser-scanning confocal microscopy. The results were compared with the appropriate passive control data and with data obtained from experiments in which the skin was exposed simply to the thermal effects induced by ultrasound treatment. A significant fraction (approximately 30%) of the intercellular lipids of the stratum corneum, which are principally responsible for skin barrier function, were removed during the application of low-frequency sonophoresis. Although the confocal images from the nile red experiments were not particularly informative, ultrasound clearly and significantly (again, relative to the corresponding controls) facilitated transport of the hydrophilic calcein via discrete permeabilized regions, whereas other areas of the barrier were apparently unaffected. Lipid removal from the stratum corneum is implicated as a factor contributing the observed permeation enhancement effects of low-frequency ultrasound [Alvarez-Roman, R. et al. Skin permeability enhancement by low frequency sonophoresis: lipid extraction and transport pathways. 2003. J Pharm Sci 92(6): p.1 138-46].
The impact of low-frequency sonophoresis appears to be much more important than that of high-frequency sonophoresis, with significant increases in transport into and from the skin following its application. Although the mechanism of action remains incompletely defined, cavitation and thermal processes are strongly implicated [Merino, G. et al. Ultrasound-enhanced transdermal transport. 2003. J Pharm Sci 92(6): p.1125-37].

In another study, application of low-frequency ultrasound was been shown to increase skin permeability, thereby facilitating delivery of macromolecules (low-frequency sonophoresis). The study sought to determine a theoretical description of transdermal transport of hydrophilic permeants induced by low-frequency sonophoresis. Parameters such as pore size distribution, absolute porosity, and dependence of effective tortuosity on solute characteristics were investigated. Pig skin was exposed to low-frequency ultrasound at 58 kHz to achieve different skin resistivities. Transdermal delivery of four permeants [mannitol, luteinizing hormone releasing hormone (LHRH), inulin, dextran] in the presence and absence of ultrasound was measured. The porous pathway model was modified to incorporate the permeant characteristics into the model and to achieve a detailed understanding of the pathways responsible for hydrophilic permeant delivery. The slopes of the log kp(p) versus log R graphs for individual solutes changed with solute molecular area, suggesting that the permeability-resistivity correlation for each permeant is related to its size. The tortuosity that a permeant experiences within the skin also depends on its size, where larger molecules experience a less tortuous path. With the modified porous pathway model, the effective tortuosities and skin porosity were calculated independently. The results of this study showed that low-frequency sonophoresis creates pathways for permeant delivery with a wide range of pore sizes. The optimum pore size utilized by solutes is related to their molecular radii [Tezel, A. et al. Description of transdermal transport of hydrophilic solutes during low-frequency sonophoresis based on a modified porous pathway model. 2003. J Pharm Sci 92(2): P.38U93].

In vitro experiments with full thickness pig skin to measure enhancements of skin conductivity and drug permeability have been performed and ultrasound was applied to pretreat the skin using a sonicator operating at a frequency of either 20 or 40 kHz. Pitting of aluminum foil was also noted to measure cavitation,
which is the principal mechanism of low-frequency sonophoresis. The skin conductivity enhancement was found to be inversely proportional to the distance of the horn from the skin. As the intensity increased, skin conductivity enhancement also increased up to a certain threshold, and then dropped off. The intensities (I(max)) at which maximum enhancement occur are about 14 W/cm2 for 20 kHz and 17 W/cm2 for 40 kHz. These findings may be useful in optimizing low-frequency sonophoresis. Overall, the dependence of transport on ultrasound parameters is similar to that of aluminum foil pitting. Hence, these results support the role of cavitation in low-frequency sonophoresis [Terahara, T. et al. Dependence of low-frequency sonophoresis on ultrasound parameters; distance of the horn and intensity. 2002. Int J Pharm 235(1-2): p.35-42].

Enhancement of drug transport via low frequency sonophoresis is thought to be mediated through cavitation, the formation and collapse of gaseous bubbles. It has been hypothesized that the efficacy of low-frequency sonophoresis can be significantly enhanced by provision of nuclei for cavitation. In a particular study, two porous resins, Diaion HP20 and Diaion HP2MG (2MG), were used as cavitation nuclei. The effect of these resins on cavitation using pitting of aluminum foil was measured. 2MG showed a higher efficacy in enhancing cavitation compared with Diaion HP20. 2MG was also effective in enhancing transdermal mannitol transport. These results confirmed that the addition of cavitation nuclei such as porous resins further increases the effect of low-frequency ultrasound on skin permeability [Terahara, T. et al. Porous resins as a cavitation enhancer for low-frequency sonophoresis. 2002. J Pharm Sci 91(3): p.753-9].

Electroporation

Electroporation is the transitory structural perturbation of lipid bilayer membranes due to the application of very short (< 1 sec) high voltage pulses. Its application to the skin has been shown to increase transdermal drug delivery by several orders of magnitude. Moreover, electroporation used alone or in combination with other enhancement methods, expands the range of drugs (small to macromolecules, lipophilic or hydrophilic, charged or neutral molecules), which can be delivered transdermally. Molecular transport through transiently permeabilized skin by electroporation results mainly from enhanced diffusion and electrophoresis.
The efficacy of transport depends on the electrical parameters and the physicochemical properties of drugs. The in vivo application of high voltage pulses is well tolerated but muscle contractions are usually induced. The electrode and patch design is an important issue to reduce the discomfort of the electrical treatment in humans [Denet, A.R. et al. Skin electroporation for transdermal and topical delivery. 2004. Adv Drug Deliv Rev 56(5): p.659-74].

Iontophoresis

Iontophoresis or ElectroMotive Drug Administration (EMDA) is a very effective method of delivering drugs to the affected site that is commonly used in many countries including the USA. Instead of injecting the drug (usually a steroid) directly into the inflamed, iontophoresis spreads a high concentration of drug evenly through the tissue applying a low density electrical current for times ranging from minutes to hours that attracts the ions in the molecules of the drug and drives them through the skin to be absorbed by the inflamed tissue.

Transdermal iontophoretic delivery of hydrocortisone solubilized in an aqueous solution of hydroxypropyl-beta-cyclodextrin (HP-beta-CyD) has been investigated and compared with chemical enhancement of co-solvent formulations [Chang, S.L. and Banga, A.K. Transdermal iontophoretic delivery of hydrocortisone from cyclodextrin solutions. 1998. J Pharm Pharmacol 50(6): p.635-40]. The passive permeation of hydrocortisone through human cadaver skin was higher when delivered from propylene glycol than when delivered after solubilization in an aqueous solution of HP-beta-CyD. However, the iontophoretic delivery of the 1% hydrocortisone-9% HP-beta-CyD solution was higher than the amount delivered passively by the 1% hydrocortisone-propylene glycol formulation, even if oleic acid was used as a chemical enhancer. Iontophoretic delivery of 1% hydrocortisone with 3% or 15% HP-beta-CyD was lower than that of the 9% HP-beta-CyD solution. These data suggest that free hydrocortisone rather than complexes is predominantly delivered iontophoretically through the skin and the HP-beta-CyD complex serves as a carrier to replenish depletion of hydrocortisone. HP-beta-CyD prevents hydrocortisone from forming a skin reservoir. Iontophoresis provides better enhancement of transdermal delivery of hydrocortisone than the chemical approach when just sufficient HP-beta-CyD is added to solubilize the hydrocortisone [Chang, S.L. and Banga, A.K.].

Penetration Enhancers

Another long-standing approach for improving transdermal drug delivery uses penetration enhancers (also called sorption promoters or accelerants), which penetrate into skin to reversibly decrease the barrier resistance. Numerous compounds have been evaluated for penetration enhancing activity, including sulphoxides (such as dimethylsulphoxide, DMSO), Azones (e.g. laurocapram), pyrrolidones (for example 2-pyrrolidone, 2P), alcohols and alkanols (ethanol, or decanol), glycols (for example propylene glycol, PG, a common excipient in topically applied dosage forms), surfactants (also common in dosage forms) and terpenes. Many potential sites and modes of action have been identified for skin penetration enhancers; the intercellular lipid matrix in which the accelerants may disrupt the packing motif, the intracellular keratin domains or through increasing drug partitioning into the tissue by acting as a solvent for the permeant within the membrane. Further potential mechanisms of action, for example with the enhancers acting on desmosomal connections between corneocytes or altering metabolic activity within the skin, or exerting an influence on the thermodynamic activity/solubility of the drug in its vehicle are also feasible [Williams, A.C. and Barry, B.W. Penetration enhancers. 2004. Adv Drug Deliv Rev 56(5): p.603-18].

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a somewhat lipophilic central cavity. Cyclodextrins are able to form water-soluble inclusion complexes with many lipophilic water-insoluble drugs. In aqueous solutions, drug molecules located in the central cavity are in a dynamic equilibrium with free drug molecules. Furthermore, lipophilic molecules in the aqueous complexation media will compete with each other for a space in the cavity. Due to their size and hydrophilicity only insignificant amounts of cyclodextrins and drug/cyclodextrin complexes are able to penetrate into lipophilic biological barriers, such as intact skin. In general, cyclodextrins enhance topical drug delivery by increasing the drug availability at the barrier surface. At the surface the drug molecules partition from the cyclodextrin cavity into the lipophilic barrier. Thus, drug delivery from aqueous cyclodextrin solutions is both diffusion controlled and

It is well known that cyclodextrins can enhance the permeation of poorly soluble drugs through biological membranes. However, the permeability will decrease if cyclodextrin is added in excess of the concentration needed to solvate the drug. The effect of cyclodextrins cannot be explained as solely due to increased solubility of the drug in the aqueous donor phase nor can it be explained by assuming that cyclodextrins act as classical permeation enhancers, i.e. by decreasing the barrier function of the lipophilic membrane. Researches have modeled the effect of cyclodextrins in terms of mixed barrier consisting of both diffusion and membrane controlled diffusion, where the diffusion of the drug in the aqueous diffusion layer is significantly slower than in the bulk of the donor. This diffusion model is described by simple mathematical equation where the properties of the system are expressed in terms of two constants P(M)/Kd and M 1/2. Data for the permeation of hydrocortisone through hairless mouse skin in the presence of various cyclodextrins, and cyclodextrin polymer mixtures, were fitted to obtain values for these two constants. The rise in flux with increased cyclodextrin complex concentration and fall with excess cyclodextrin was accurately predicted. Data for the permeation of drugs through semi-permeable cellophane membrane could also be fitted to the equation. It was concluded that cyclodextrins act as permeation enhancers carrying the drug through the aqueous barrier, from the bulk solution towards the lipophilic surface of biological membranes, where the drug molecules partition from the complex into the lipophilic membrane [Masson, M. et al. Cyclodextrins as permeation enhancers: some theoretical evaluations and in vitro testing. 1999. J Control Release 59(1): p.107-18].

Example 1

Isolation and identification of FL3P:
The following assays were performed in order to verify that fructose-lysine (FL) could be identified in its phosphorylated state, e.g., FL3P. A 31P NMR analysis of a perchloric acid extract of diabetic rat kidneys was performed and showed a new sugar monophosphate resonance at 6.24 ppm which is not observed in non-kidney tissue and is present at greatly reduced levels in non-diabetic kidney. The
The compound responsible for the observed resonance was isolated by chromatography of the extract on a macrocrystalline cellulose column using 1-butanol-acetic acid-water (5:2:3) as eluent. The structure was determined by proton 2D COSY to be fructose-lysine 3-phosphate. This was later confirmed by injecting animals with FL, prepared as previously described (Finot and Mauson, 1969, Helv. Chim. Acta, 52:1488), and showing direct phosphorylation to FL3P.

Using FL specifically deuterated in position-3 confirmed the position of the phosphate at carbon-3. This was performed by analyzing the $^{31}$P NMR spectra, both coupled and decoupled. The normal P-O-C-H coupling produces a doublet in FL3P with a J value of 10.3 Hz; whereas P-O-C-D has no coupling and produces a singlet both coupled and decoupled, as was found for 3-deuterated FL3P. A unique property of FL3P is that when treated with sodium borohydride it is converted into two new resonances at 5.85 and 5.95 ppm, which correspond to mannitol and sorbitol-lysine 3-phosphates.

Example 2

**Synthesis of FL3P:**

1 mmol of dibenzyl-glucose 3-phosphate and 0.25 mmol of α-carbobenzoxy-lysine was refluxed in 50 ml of MeOH for 3 hours. The solution was diluted with 100 ml water and chromatographed on a Dow-50 column (2.5 x 20 cm) in the pyridinium form and eluted first with water (200 ml) and then with 600 ml buffer (0.1M pyridine and 0.3M acetic acid). The target compound eluted at the end of the water wash and the beginning of the buffer wash. The results demonstrated that removal of the cbz and benzyl blocking groups with 5% Pd/C at 20 psi of hydrogen gave FL3P in 6% yield.

Example 3

**Enzymatic production of FL3P from FL and ATP and assay for screening inhibitors:**

Initially $^{31}$P NMR was used to demonstrate kinase activity in the kidney cortex. A 3 g sample of fresh pig kidney cortex was homogenized in 9 ml of 50 nM Tris-HCl containing 150 mM KCl, 5 mM DTT, 15 mM MgCl$_2$, pH 7.5. This was centrifuged at 10,000 g for 30 minutes, and then the supernatant was centrifuged at 100,000 g for 60 minutes. Ammonium sulfate was added to 60% saturation. After
1 hour at 4°C the precipitate was collected by centrifugation and dissolved in 5 ml of original buffer. A 2 ml aliquot of this solution was incubated with 10 mM ATP and 10 mM of FL (prepared as in Example 1, above) for 2 hours at 37°C. The reaction was quenched with 300 µl of perchloric acid, centrifuged to remove protein, and desalted on a column of Sephadex G 10 (5 x 10 cm). 31P NMR analysis of the reaction mixture detected formation of FL3P.

Based on the proof of kinase activity thus obtained, a radioactive assay was developed. This assay was designed to take advantage of the binding to Dow-50 cation exchange resin by FL3P. This characteristic of FL3P was discovered during efforts to isolate it. Since most phosphates do not bind to this resin, it was suspected that the bulk of all compounds that react with ATP as well as any excess ATP would not be bound. The first step was to determine the amount of resin required to remove the ATP in the assay. This was accomplished by pipetting the mixture into a suspension of 200 mg of Dow-1 in 0.9 ml H2O, vortexing, and centrifuging to pack the resin. From this 0.8 ml of supernatant was pipetted onto 200 mg of fresh dry resin, vortexed and centrifuged. A 0.5 ml volume of supernatant was pipetted into 10 ml of Ecoscint A and counted. Residual counts were 85 cpm. This procedure was used for the assay. The precipitate from 60% ammonium sulfate precipitation of the crude cortex homogenate was redissolved in the homogenate buffer at 4°C. The assay contains 10 mM γ32P-ATP (40,000 cpm), 10 mM FL, 150 mM KCl, 15 mM MgCl2, 5 mM DTT in 0.1 ml of 50 mM TrisHCl, pH 7.5. The relationship between rates of FL3P production and enzyme concentration was determined using triplicate determinations with 1, 2, and 4 mg of protein for 30 minutes at 37°C. Blanks run concurrently without FL were subtracted and the data recorded. The observed activity corresponds to an approximate FL3P synthesis rate of 20 nmols/hr/mg protein.

Example 4

Inhibition of the formation of 3-deoxyglucosone by meglumine and various polvollvsines:

a. General polvollysine synthesis:

The sugar (11 mmol), α-carbocenzyx-lysine (10 mmols) and NaBH3CN (15 mmoles) were dissolved in 50 ml ofMeOH-H2O (3:2) and stirred at 25°C for 18 hours. The solution was treated with an excess of Dow-50 (H) ion exchange resin to decompose excess NaBH3CN. This mixture (liquid plus resin) was
transferred onto a Dow-50 (H) column (2.5 x 15 cm) and washed well with water to remove excess sugar and boric acid. The carbobenzoxy-polyollysine was eluted with 5% NH₄OH. The residue obtained upon evaporation was dissolved in water-methanol (9:1) and reduced with hydrogen gas (20 psi) using a 10% palladium on charcoal catalyst. Filtration and evaporation yields the polyollysine.

b. Experimental protocol for reduction of urinary and plasma 3-deoxyglucosone by sorbitollysine, mannitollysine and galactitollysine:

Urine was collected from six rats for three hours. A plasma sample was also obtained. The animals were then given 10 µmols of either sorbitollysine, mannitollysine, or galactitollysine by intraperitoneal injection. Urine was collected for another three hours, and a plasma sample obtained at the end of the three hours.

a. 3-deoxyglucosone was measured in the samples, as described in Example 5, below, and variable volumes were normalized to creatinine. The average reduction of urinary 3-deoxyglucosone was 50% by sorbitollysine, 35% by mannitollysine and 35% by galactitollysine. Plasma 3-deoxyglucosone was reduced 40% by sorbitollysine, 58% by mannitollysine and 50% by galactitollysine.

b. Use of meglumine to reduce urinary 3-deoxyglucosone:

Three rats were treated as in b), immediately above, except meglumine (100 µmols) was injected intraperitoneally instead of the above-mentioned lysine derivatives. Three hours after the injection the average 3-deoxyglucosone concentrations in the urine were decreased 42%.

Example 5

Elevation of urinary FL, 3DG and 3DF in humans following ingestion of glycated protein:

a. Preparation of glycated protein containing food product:

260 g of casein, 120 g of glucose and 720 ml of water were mixed to give a homogeneous mixture. This mixture was transferred to a metal plate and heated at 65°C for 68 hours. The resulting cake was then pulverized to a coarse powder.

This powder contained 60% protein as determined by the Kjeldahl procedure.

b. Measurement of glycated lysine content:
One gram of the powder prepared as in step a., above, was hydrolyzed by refluxing with 6N HCl for 20 hours. The resulting solution was adjusted to pH 1.8 with NaOH solution and diluted to 100 ml. The fructoselysine content was measured on an amino acid analyzer as furosine, the product obtained from acid hydrolysis of fructoselysine. In this way, it was determined that the cake contained 5.5% (w/w) fructoselysine.

c. Experimental protocol:

Volunteers spent two days on a fructoselysine-free diet and then consumed 22.5 g of the food product prepared as described herein, thus effectively receiving a 2 gram dose of fructoselysine. Urine was collected at 2 hour intervals for 14 hours and a final collection was made at 24 hours.

d. Measurement of FL, 3DG and 3DF in urine:

FL was measured by HPLC with a Waters 996 diode Array using a Waters C18 Free Amino Acid column at 46°C and a gradient elution system of acetonitrile-methyl alcohol-water (45:15:40) into-acetonitrile-sodium acetate-water (6:2:92) at 1 ml/min. Quantitation employed an internal standard of meglumine.

3DF was measured by HPLC after deionization of the sample. Analyses were performed on a Dionex DX-500 HPLC system employing a PA1 column (Dionex) and eluting with 32 mM sodium hydroxide at 1 ml/min. Quantitation was performed from standard curves obtained daily with synthetic 3DF.

3DG was measured by GC-MS after deionization of the sample. 3DG was derivatized with a 10-fold excess of diaminonaphthalene in PBS. Ethyl acetate extraction gave a salt free fraction which was converted to the trimethyl silyl ethers with Tri-Sil (Pierce). Analysis was performed on a Hewlett-Packard 5890 selected ion monitoring GC-MS system. GC was performed on a fused silica capillary column (DB-5,25 mx.25 mm) using the following temperature program: injector port 250°C, initial column temperature 150°C which is held for 1 minute, then increased to 290°C at 16°C/minute and held for 15 minutes. Quantitation of 3DG employed selected ion monitoring using an internal standard of U-13C-3DG.

The results of the experiments described in this example are now presented.

The graph depicted in Figure 3 represents production of FL, 3DF, and 3DG in the urine of one volunteer after consuming the glycated protein. The rapid
appearance of all three metabolites is clearly evident. Both 3DF and 3DG show a slight elevation even after twenty-four hours.

The graph shown in Figure 4 represents the formation of 3DF in each of the members of a seven-person test group. A similar pattern was seen in all cases. As demonstrated in Figure 4, 3DF excretion peaks about 4 hours after the FL bolus and a slight elevation of 3DF is noticeable even 24 h after the bolus.

Example 6

Effects of increased dietary uptake of glycated proteins:

N-acetyl-β-glucosaminidase (NAGase) is an enzyme excreted into the urine in elevated concentration in diabetics. It is thought to be an early marker of tubular damage, but the pathogenesis of increased NAGase in urine is not well understood. The increased urinary output of NAGase in diabetics has been proposed to be due to activation of lysosomes in proximal tubules induced by diabetes with an increased output into the urine rather than destruction of cells.

Rats were fed a diet containing 0.3% glycated protein or control feed over several months. The urinary output of NAGase and 3DF were determined at various times, as indicated in Figure 5. The amount of 3DG excreted in urine was also determined.

The results obtained in this example demonstrate that in all comparisons 3DF and NAGase levels are elevated in the experimental group relative to the control. Thus, animals fed glycated protein excrete excess NAGase into their urine, similar to results obtained with diabetics. NAGase output increased by approximately 50% in the experimental group, compared with control animals. The experimental animals also had a five-fold increase in urine 3DF compared with controls. Urinary 3DF was found to correlate extremely well with 3DG, as can be seen in Figures 5 and 6.

Example 7

Electrophoretic analysis of kidney proteins:

Two rats were injected daily with 5 µmols of either FL or mannitol (used as a control) for 5 days. The animals were sacrificed and the kidneys removed and dissected into the cortex and medulla. Tissues were homogenized in 5 volumes of 50 mM Tris-HCl containing 150 mM KCl, 15 mM MgCl2 and 5 mM DTT, pH 7.5.
Cellular debris was removed by centrifugation at 10,000 x g for 15 minutes, and the supernatant was then centrifuged at 150,000 x g for 70 minutes. The soluble proteins were analyzed by SDS PAGE on 12% polyacrylamide gels as well as on 4-15 and 10-20% gradient gels.

It was found that in all cases, lower molecular weight bands were missing or visually reduced from the kidney extract of the animal injected with FL when compared with the animal injected with mannitol.

Example 8

Synthesis of 3-O-rnethylisorbitolylsine (Structure XDQ)

3-OMe glucose (25 grams, 129 mmol) and α-Cbz-lysine (12 grams, 43 mmol) were dissolved in 200 ml of water-methanol (2:1). Sodium cyanoborohydride (10 grams, 162 mmol) was added and the reaction stirred for 18 days at room temperature. Reaction of α-Cbz-lysine was monitored by thin layer chromatography on silica gel employing 1-butanol-acetic acid-water (4:1:1) using ninhydrin for visualization. The reaction was complete when no α-Cbz-lysine remained. The solution was adjusted to pH 2 with HCl to decompose excess cyanoborohydride, neutralized and then applied to a column (5x50 cm) of Dowex-50 (H+) and the column washed well with water to remove excess 3-O-me-glucose. The target compound was eluted with 5% ammonium hydroxide. After evaporation the residue was dissolved in 50 ml of water-methanol (2:1) and 10% Pd/C (0.5 gram) was added. The mixture was shaken under 20 psi of hydrogen for 1 hr. The charcoal was filtered off and the filtrate evaporated to a white powder (10.7 gram, 77% yield based on α-Cbz-lysine) that was homogeneous when analyzed by reversed phase HPLC as the phenylisothiocyanate derivative. Elemental analysis: Calculated for
\[
C_{13}H_{28}N_2O_7CH_3OH \cdot 2H_2OC, \ 42.86; \ H, \ 9.18; \ N, \ 7.14. \ \text{Found:} \ C, \ 42.94; \ H, \ 8.50; \ N, \ 6.95.
\]

Other specific compounds having the structure of formula (XIX), above, may be made, e.g., by glycation of a selected nitrogen- or oxygen-containing starting material, which may be an amino acid, polyaminoacid, peptide or the like, with a glycating agent, such as fructose, which may be chemically modified, if desired, according to procedures well know to those skilled in the art.

Example 9
Additional assay for FL3P kinase activity:

a. Preparation of Stock Solutions:

An assay buffer solution was prepared which was 100 mM HEPES pH 8.0, 10 mM ATP, 2 mM MgCl₂, 5 mM DTT, 0.5 mM PMSF. A fructosyl-spermine stock solution was prepared which was 2 mM fructosyl-spermine HCl. A spermine control solution was prepared which was 2 mM spermine HCl.

b. Synthesis of Fructosyl-spermine:

Synthesis of fructosyl-spermine was performed by an adaptation of a known procedure (J. Hodge and B. Fisher, 1963, Methods Carbohydr. Chem., 2:99-107). A mixture of spermine (500 mg), glucose (500 mg), and sodium pyrosulfite (80 mg) was prepared in a molar ratio of 8:4:1 (spermine:glucose:pyrosulfite) in 50 ml of methanol-water (1:1) and refluxed for 12 hours. The product was diluted to 200 ml with water and loaded onto a DOW-50 column (5 x 90 cm). The unreacted glucose was removed by 2 column volumes of water and the product and unreacted spermine were removed with 0.1 M NH₄OH. Pooled peak fractions of the product were lyophilized and concentration of fructosyl-spermine was determined by measuring the integral of the C-2 fructosyl peak in a quantitative ¹³C NMR spectrum of the product (NMR data collected with a 45° pulse, a 10 second relaxation delay and without NOE decoupling).

c. Kinase Assay to Determine Purification:

An incubation mixture was prepared including 10 µl of the enzyme preparation, 10 µl of assay buffer, 1.0 µCi [³²P] ATP, 10 µl of fructosyl-spermine stock solution and 70 µl of water and incubated at 37°C for 1 hour. At the end of the incubation 90 µl (2 x 45 µl) of the sample was spotted onto two 2.5 cm diameter cellulose phosphate disks (Whatman P-81) and allowed to dry. The disks were washed extensively with water. After drying, the disks were placed in scintillation vials and counted.

Each enzyme fraction was assayed in duplicate with an appropriate spermine control.

Example 10

Kidney pathology observed in test animals on glycated protein diet:

Three rats were maintained on a glycated protein diet (20% total protein; 3% glycated) for 8 months and compared to 9 rats of the same age maintained
on a control diet. The glycated protein diet consisted of a standard nutritious diet to which 3% glycated protein had been substituted for nonglycated protein. The glycated protein was made by mixing together casein and glucose (2:1), adding water (2X the weight of the dried material), and baking the mixture at 60°C for 72 hours. The control was prepared in the same way except that no water was used and the casein and glucose were not mixed prior to baking.

The primary finding was a substantial increase in damaged glomeruli in the animals on the glycated diet. Typical lesions observed in these animals were segmental sclerosis of the glomerular tuft with adhesion to Bowman's capsule, tubular metaplasia of the parietal epithelium and interstitial fibrosis. Animals on the glycated protein diet, and only one of the animals on the control diet showed more than 13% damaged glomeruli. The probability of this happening by chance is less than 2%. In addition to the pathological changes observed in the glomeruli, a number of hyalinated casts within tubules were observed. More of these hyalinated casts were found in animals on the glycated diet, although these were not quantitated. Increased levels of NAGase were also observed in the animals on the glycated diet.

Based on the results of this experiment, the glycated diet appeared to cause the test animals to develop a series of histological lesions similar to those seen in the diabetic kidney.

Example 12

Carcinogenic effects of fructoselysine pathway:

To investigate the carcinogenic potential of metabolites formed in the fructoselysine pathway, experiments were conducted on a strain of rats with a high susceptibility to kidney carcinomas.

Four rats were put on a glycated protein diet and three rats on a control diet. After ten weeks on the diet, the animals were sacrificed and their kidneys examined.

In all four animals on the diet, kidney carcinomas of size greater than 1 mm were found, whereas no lesions this large were found in the control animals. The probability of this happening by chance is less than 2%.

The data demonstrate that there are elevated 3DG levels, caused by the excess fructoselysine coming from the glycated protein in the diet, in the kidney tubular cells (known to be the cell of origin of most kidney carcinomas), and the 3DG
can interact with the cellular DNA, leading to a variety of mutagenic and ultimately carcinogenic events. The possibility exists that this process is important in the development of human cancers in the kidney and elsewhere.

Example 13

Dietary effects of glycated protein diet on renal cell carcinoma in susceptible rats:

In addition to the experiments described above, experiments were performed to assess the relationship between a glycated protein diet and renal cell carcinoma.

Twenty-eight rats with a mutation making them susceptible to the development of kidney carcinoma were divided into two cohorts. One cohort was fed a glycated protein diet and the other cohort was on a control diet. The glycated protein diet consisted of a standard nutritious diet to which 3% glycated protein had been added. The glycated protein was made by mixing together casein and glucose (2:1), adding water (2X the weight of the dried material), and baking the mixture at 60°C for 72 hours. The control was prepared in the same way except that no water was used and the casein and glucose were not mixed prior to baking. Rats were placed on the diets immediately following weaning at three weeks of age and maintained on the diets ad libitum for the next 16 weeks. The animals were then sacrificed, the kidneys fixed, and hematoxylin and eosin sections were prepared.

The histological samples were examined by a pathologist. Four types of lesions were identified. These include: cysts; very small collections of tumor-like cells, typically less than 10 cells; small tumors, 0.5 mm or less; and tumors greater than 0.5 mm. For the four types of lesions, more lesions were observed in the animals on the glycated diet than on the control diet, as shown in the following table (Table A).

<table>
<thead>
<tr>
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<th>CYSTS</th>
<th>≤ 10 CELLS</th>
<th>≤ 0.5 mm</th>
<th>&gt; 0.5 mm</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>2</td>
<td>9</td>
<td>9</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>GLYCATED</td>
<td>9</td>
<td>21</td>
<td>32</td>
<td>6</td>
<td>68</td>
</tr>
</tbody>
</table>

To summarize the results, the average number of lesions per kidney section was computed for each diet. These were 0.82 ± 0.74 and 2.43 ± 2.33 in the
control and glycated diet, respectively. The likelihood of this happening by chance is about 2 in 100,000.

These results provide strong support for the premise that the effects of the lysine recovery pathway, the discovery of which underlies the present invention, extend to causing mutations, and thus produce a carcinogenic effect as well. These results provide a basis for the development of therapeutic methods and agents to inhibit this pathway in order to reduce cancer in the kidney as well as in other organs where this pathway may have similar effects.

Example 14

Urinary excretion of 3-deoxy-fructose is indicative of progression to microalbuminuria in patients with type I diabetes.

As set forth herein, serum levels of the glycation intermediate, three deoxy-glucosone (3DG) and its reductive detoxification product, three deoxy-fructose (3DF), are elevated in diabetes. The relationship between baseline levels of these compounds and subsequent progression of microalbuminuria (MA) has been examined in a group of 39 individuals from a prospective cohort of patients at the Joslin Diabetes Center with insulin-dependent diabetes mellitus (IDDM) and microalbuminuria (based on multiple measurements during the two years of baseline starting between 1990-1993) and not on ACE inhibitors.

Baseline levels of 3DF and 3DG in random spot urines were measured by HPLC and GC-MS. Individuals that progressed to either a higher level of MA or proteinuria in the next four years (n=24) had significantly higher baseline levels of log 3DF/urine creatinine ratios compared to non-progressors (n=15) (p=0.02).

Baseline levels determined in this study were approximately 0.24 μmole/mg of creatinine in the progressors vs. approximately 0.18 μmole/mg of creatinine ratios in the non-progressors. Baseline 3DG/urine creatinine ratios did not differ between the groups. Adjustment of the baseline level of HgA1c (the major fraction of glycosylated hemoglobin) did not substantially alter these findings. These results provide additional evidence of the association between urinary 3DF and progression of kidney complications on diabetes.

a. Quantification of 3-deoxyfructose:

Samples were processed by passing a 0.3 ml aliquot of the test sample through an ion-exchange column containing 0.15 ml of AG 1-X8 and 0.15 ml of AG
5OW-X8 resins. The columns were then washed twice with 0.3 ml deionized water, aspirated to remove free liquid and filtered through a 0.45 mm Millipore filter.

Injections (50 µl) of the treated samples were analyzed using a Dionex DX 500 chromatography system. A carbopac PA1 anion-exchange column was employed with an eluant consisting of 16% sodium hydroxide (200 mM) and 84% deionized water. 3DF was detected electrochemically using a pulsed amperometric detector. Standard 3DF solutions spanning the anticipated 3DF concentrations were run both before and after each unknown sample.

b. Measurement of urine creatinine:

Urine creatinine concentrations were determined by the end-point colorimetric method (Sigma Diagnostic kit 555-A) modified for use with a plate reader. Creatinine concentrations were assessed to normalize urine volumes for measuring metabolite levels present therein.

c. Measurement of albumin in the urine:

To assess albumin levels in the urine of the test subjects, spot urines were collected and immunoelectrophoresis performed on a BN 100 apparatus with the N-albumin kit (Behring). Anti-albumin antibodies are commercially available. Albumin levels in urine may be assessed by any suitable assay including but not limited to ELISA assays, radioimmunoassays, Western, and dot blotting.

Based on the data obtained in the study of the Joslin Diabetes Center patients, it appears that elevated levels of urinary 3DF are associated with progression to microalbuminuria in diabetes. This observation provides a new diagnostic parameter for assessing the likelihood of progression to serious kidney complications in patients afflicted with diabetes.

Example 15

3-O-methyl sorbitollysine lowers systemic levels of 3DG in normal and diabetic rats:

A cohort of twelve diabetic rats was divided into two groups of six. The first group received saline-only injections, and the second received injections of 3-O-methyl sorbitollysine (50 mg/kg body weight) in saline solution. The same procedure was conducted on a cohort of twelve non-diabetic rats.
As summarized in Table B, within one week, the 3-O-methyl sorbitolylsine treatment significantly reduced plasma 3DG levels as compared to the respective saline controls in both diabetic and non-diabetic rats.

TABLE B. 3-O-Methyl sorbitolylsine (3-OMe) reduces plasma 3DG levels in diabetic and non-diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>Diabetic rats</th>
<th>Non-diabetic rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline only</td>
<td>0.94±0.28 uM</td>
<td>0.23±0.07 uM</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(n = 6)</td>
<td></td>
</tr>
<tr>
<td>3-OMe</td>
<td>0.44±0.10 uM</td>
<td>0.13±0.02 uM</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(n = 7)</td>
<td></td>
</tr>
<tr>
<td>% Reduction</td>
<td>53%</td>
<td>43%</td>
</tr>
<tr>
<td>t-test</td>
<td>p = 0.0006</td>
<td>p = 0.0024</td>
</tr>
</tbody>
</table>

The ability of 3-O-methyl sorbitolylsine to reduce systemic 3DG levels suggests that diabetic complications other than nephropathy (e.g., retinopathy and stiffening of the aorta) may also be controllable by amadorase inhibitor therapy.

Example 16

Locus of 3-O-methyl sorbitolylsine uptake in vivo is the kidney:

Six rats were injected intraperitoneally with 13.5 nmols (4.4 mg) of 3-O-methyl sorbitolylsine. Urine was collected for 3 hours, after which the rats were sacrificed. The tissues to be analyzed were removed and freeze clamped in liquid nitrogen. Perchloric acid extracts of the tissues were used for metabolite analysis. The tissues examined were taken from the brain, heart, muscle, sciatic nerve, spleen, pancreas, liver, and kidney. Plasma was also analyzed.

The only tissue extract found to contain 3-O-methyl sorbitolylsine was that of the kidney. The urine also contained 3-O-methyl sorbitolylsine, but plasma did not. The percentage of the injected dose recovered from urine and kidney varied between 39 and 96%, as shown in Table C, below.

TABLE C.
Example 17

Amadorase/fructosamine kinase activity accounts for a majority of 3DG production:

Enzymatic production of 3DG was demonstrated in an *in vitro* assay with various key components (10 mM Mg-ATP, partially purified amadorase, 2.6 mM FL) omitted from the reaction in order to assess their importance in 3DG production.

The results show that 3DG production is 20-fold higher in the presence of kidney extract containing amadorase and its substrates (compare Table D, reactions 1 and 3). Clearly, the vast majority of 3DG production is enzymatically mediated in the presence of amadorase.

**TABLE D.** Amadorase-dependent production of 3DG after 24 hours

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Amadorase</th>
<th>ATP</th>
<th>FL (mM)</th>
<th>FL3P (mM)</th>
<th>3DG (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>2.6</td>
<td>0.2</td>
<td>1.58</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>2.6</td>
<td>0</td>
<td>0.08</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
<td>2.6</td>
<td>0</td>
<td>0.09</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>2.6</td>
<td>0</td>
<td>0.08</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Example 18

**Effects of 3DG, and inhibition of 3PG, on collagen crosslinking:**

Collagen is present at high levels in skin. To this end, it was determined what effect 3DG has on collagen crosslinking.

Collagen I was incubated in the presence or absence of 3DG *in vitro*. Calfskin collagen Type I (1.3 mg; Sigma) was incubated in 20 mM Na-phosphate buffer, pH 7.25, either alone, with 5 mM 3DG, or with 5 mM 3DG plus 10 mM arginine, in a total volume of 1 ml at 37°C for 24 hours and then frozen and lyophilized. The residue was dissolved in 0.5 ml of 70% formic acid and cyanogen bromide was added (20:1, w/w). This solution was incubated at 30°C for 18 hours. Samples were dialyzed against 0.125 M Tris, pH 6.8, containing 2% SDS and 2% glycerol, in dialysis tubing with a molecular weight cutoff of 10,000. The samples were all adjusted to a volume of 1 ml. The extent of collagen crosslinking was determined by applying equal volumes of sample and analyzing by SDS-PAGE electrophoresis (16.5% Tris-tricine gel), as determined by the effects of 3DG on the migration of collagen.

It was found that treatment of collagen with 3DG caused the collagen to migrate as if it had a higher molecular weight, which is indicative of crosslinking.

The image of the silver-stained gel in Figure 12 demonstrates that there are fewer high molecular bands in the groups containing collagen alone or collagen plus 3DG plus arginine. There are more high molecular weight bands in the group treated with 3DG, in the absence of a 3DG inhibitor. There appears to be more protein in the sample treated with 3DG alone. Because all three samples started with the same mount of protein, without being bound by theory, it can be concluded that during dialysis fewer peptides escaped from the 3DG treated sample because more crosslinks were produced and higher molecular weight proteins were retained. In other words, there appears to be less protein in the control and 3DG plus arginine groups, because smaller molecular peptides diffused out during dialysis.

Example 19

**Localization of 3DG in Skin:**

The invention as described in the present disclosure identifies for the first time the presence of 3DG in skin.
A mouse skin model was used. One centimeter (1 cm) squares of skin were prepared and subjected to extraction with perchloric acid. 3DG was measured as described above. Six mice were used and the average amount of 3DG detected in the skin was 1.46 +/- 0.3 µM. This value was substantially higher than the plasma concentrations of 3DG detected in the same animals (0.19 +/- 0.05 µM). These data, and the data described below in Example 20, suggest that the high levels of 3DG in the skin are due to production of 3DG in the skin.

Example 20

Localization of Amadorase mRNA in Skin:

Although high levels of 3DG were found in skin (see previous Example), it was not known whether the 3DG was formed locally and whether skin had the ability to produce 3DG enzymatically. The presence of amadorase mRNA was analyzed and was utilized as one measure of the ability of skin to produce the 3DG present in skin (see previous example).

PolyA+ messenger RNA isolated from human kidney and skin was purchased from Stratagene. The mRNA was used in RT-PCR procedures. Using the published sequence for amadorase (Delpierre et al., 2000, Diabetes 49:10:1627-1634; Szwergold et al., 2001, Diabetes 50:2139-2147), a reverse primer to the 3' terminal end of the gene (bp 930-912) was subjected to RT to create a cDNA template for PCR. This same primer was used along with a forward primer from the middle of the amadorase gene (bp 412-431) to amplify the amadorase gene from the cDNA template. The product of the PCR should be a 519 bp fragment. Human skin and kidney samples were subjected to RT-PCR and analyzed by agarose gel electrophoresis, as were controls which contained no cDNA templates.

The results demonstrate that skin does indeed express amadorase mRNA. Subsequent expression of the protein would account for production of 3DG in skin. As expected, a 519 bp product was observed (see Figure 13). Not only was the 519 bp fragment found in kidney (lane 1), it was also found in skin (lane 3). The 519 bp fragment was not detected in the groups which received no cDNA template (lanes 2 and 4).

Example 21

Effects of Fructoselvsine on kidney cells in vitro:
As described above, a diet high in glycated proteins, e.g., fructoselysine, has a profound effect on metabolism in vivo. Therefore, the effects of fructoselysine were tested directly on kidney cells in vitro.

The results demonstrate that fructoselysine administered to kidney cells in vitro causes an increase in type IV collagen levels in the cells. Type IV collagen production was measured in mouse mesangial cells. Controls (grown with 10% glucose) produced 300 ng of Type IV collagen per 10,000 cells, whereas fructoselysine treated cells (5 or 10 mM fructoselysine with 10 mM glucose) produced 560 and 1100 ng/10,000 cells.

Example 22
Inhibition of 3DG by inhibiting Amadorase mRNA and protein:
3DG synthesis may be inhibited by inhibiting the components of the enzymatic pathway leading to its synthesis. This can be done in several ways. For example, the enzyme which leads to the synthesis of 3DG, called amadorase herein (a fructosamine-3-kinase) can be inhibited from acting using a compound as described above, but it can also be inhibited by blocking the synthesis of its message or protein or by blocking the protein itself, other than with a compound, as described above.

Amadorase mRNA and protein synthesis and function may be inhibited using compounds or molecules such as transcription or translation inhibitors, antibodies, antisense messages or oligonucleotides, or competitive inhibitors.

Nucleic Acid and Protein Sequences
The following represents the 988 bp mRNA-derived DNA sequence for amadorase (fructosamine-3-kinase), Accession No. NM_022158 (SEQ ID NO:1) (see Figure 10):

```
cgtaagctt ggacagagcc catggagcac tgcctgccg ccagctgcc caccgegcc  
cctgcgggcct tcggcggccc cggcgccggc tgcatcagcg agggccgagc ctacgacacg  
gacgcaggcc cagtgttcgt caaagtcaac gcaggaacgc aggccggca gatgtttgag  
rgggaggtgg cgacgctgga ggcctcggc agcaaggggc tgggtgcgggt gcggagccc  
atgaagctca tcagcctgcc ggagggggc gaggagttcg ggcgcctttg tggagata  
agagcttga gcagtcaagc atcaaaactt ggagagcaga tggcagattt gcatctttac  
agacgacggc tgccttgaga ctacgaagc gtcgctcgc gtcggacttc  
```
The following represents the 309 amino acid residue sequence of human amadorase (fructosamine-3-kinase), Accession No. NP_071441 (SEQ ID NO:2) (see Figure 11):

```
1     meqllraelr tatlrafggp gagcisegra ydtdagpvf vknrqtarq mfgevasle
61    alrstglrvr prpmkvidlp gggafvmehe lmksllsqa sklgeqmadi hlynqklrek
121   lkeeentvgr rgegaepqyv dkfgfhtvte cgfpqvnem qddwtffar hrIqaqldli
181   ekdyadrear elwsrlqvki pdrfgleiv pullhgdwss gnvaedvypg iyypasfgy
241   hsefelalal mffgfprssf tayhrikpa pgfdqrlly qlfynlhnw hfgreyrsps
301   lgtmrrllk
```

The sequences identified above were submitted by Delpierre et al. (2000, Diabetes 49: 16227-1634). The sequence data of Szwergold et al. (2001, Diabetes 50:2139-2147) are in excellent agreement with those of Delpierre et al. For example, the protein sequence deduced by Szwergold et al. (2001, Diabetes 50:2139-2147) is identical with the cloned human fructosamine-3-kinase sequence of Delpierre et al. (2000, Diabetes 49:16227-1634) in 307 of 309 amino acid residues. Thus, reliance on the published sequences of either group should not be a problem, however, to ensure that no problems arise when a sequence of the protein is to be used, only those portions of the sequence which are not different between the two published sequences will be used.

Example 23

Presence of Alpha-Dicarbonyl Sugars in Sweat
As disclosed herein, alpha-dicarbonyl sugars are present in skin, but their presence in sweat had not been determined. One of the functions of skin is to act as an excretory organ, therefore, it was determined whether alpha-dicarbonyl sugars are excreted in sweat.

Samples of human sweat were analyzed for the presence of 3DG, as described above. Samples from four subjects were obtained and 3DG was determined to be present at levels of 0.189, 2.8, 0.312, and 0.11 µM, respectively. Therefore, the results demonstrate the presence of 3DG in sweat.

Example 24

**Effects of DYN 12 (3-O-methylsorbitol) vs (3-O-methylsorbitol) on Skin Elasticity**


Experiments were performed to determine the effects of DYN 12 on the loss of skin elasticity associated with diabetes. To this end, two groups of STZ-diabetic rats and two groups of normal rats were subjected to treatment with DYN 12 or saline. One group of STZ-diabetic rats (n=9) received daily subcutaneous injections of DYN 12 at 50 mg/kg for eight weeks, as did one group of normal rats (n=6). A group of control diabetic rats (n=10) and a group of normal rats (n=6) received saline instead of DYN 12. One rat was removed from the diabetic DYN 12 group after 2 weeks because its blood glucose readings were inconsistent (too low) with other diabetic rats.

A non-invasive procedure based on CyberDERM, Inc. technology utilizing a skin elasticity measurement device was used to test the effects of DYN 12 treatment on skin elasticity. The procedure provides for non-invasive measurement of skin elasticity based upon the amount of vacuum pull required to displace skin. A suction cup probe is adhered to an area of shaved skin in order to form an airtight seal. Then, a vacuum is applied to the area of the skin inside the suction cup until the skin is displaced past a sensor located inside the probe. Accordingly, the more pressure that is required to displace the skin, the less elastic the skin is.

The data demonstrate that after eight weeks of treatment skin elasticity in diabetic rats treated with DYN 12 was greater than skin elasticity in diabetic
animals which were treated with saline. As seen in Figure 14, the amount of pressure
needed to displace the skin of diabetic rats treated with saline (7.2 +/- 3.0 kPA) was
approximately 2 to 2.25 fold higher than the pressure needed to displace the skin of
diabetic animals treated with DYN 12 (3.2 +/- 1.2 kPA). Also, the elasticity value
observed in diabetic rats treated with DYN 12 was not statistically different from the
value found in non-diabetic rats treated with saline (p = 0.39) (Table E). Thus, the
result of treatment of diabetic animals with DYN 12, an indirect inhibitor of 3DG,
was skin with greater elasticity than skin in diabetic animals which received only
saline.

Table E. Statistical Analysis and Comparison of Cohort Groups.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic saline</td>
<td>Non-diabetic saline</td>
<td>p = 0.01</td>
</tr>
<tr>
<td>Diabetic saline</td>
<td>Diabetic DYN 12</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>Diabetic saline</td>
<td>Non-diabetic DYN 12</td>
<td>p = 0.003</td>
</tr>
<tr>
<td>Diabetic DYN 12</td>
<td>Non-diabetic DYN 12</td>
<td>p = 0.39</td>
</tr>
<tr>
<td>Non-diabetic saline</td>
<td>Non-diabetic DYN 12</td>
<td>p = 0.26</td>
</tr>
</tbody>
</table>

The above data demonstrate that the administration of DYN 12 to
diabetic rats prevents the loss of skin elasticity (e.g., sclerosis and thickening of the
basement membrane of the skin) that is typically observed in untreated diabetic rats,
which is evidence that the excess 3DG found in diabetics is the cause of the loss of
elasticity. The data disclosed herein further indicate that reducing 3DG levels can also
serve to maintain skin elasticity in normal individuals.

Skin elasticity measurements were also taken on the test subjects as
described above, but without sedating the test animals before measurement. Figure 15
illustrates skin elasticity measurements taken on the hind leg of the test subjects while
the subjects were alert and being restrained by a technician.

In these experiments, the animals were fiercely fighting restraint and
the results are different. The diabetic animals without drug treatment showed less
ability to "pull away" from the suction cup and therefore show less "resistance to pull". On the other hand, both the diabetic animals receiving drug and the normal
animals had a greater capacity to pull away from the suction cup, and both groups of
animals demonstrated stiffness and greater muscle tension. This indicates that the inhibition of the enzyme, and most likely, inactivation of 3DG, results in the sparing of microcirculation deterioration and neuro-deterioration that typifies the diabetic condition.

Example 25

Level of 3DG in scleroderma skin

It has been determined, according to the methods disclosed previously elsewhere herein, that normal skin had the following concentrations of 3DG (data from several subjects): 0.9 µM, 0.7 µM, and 0.6 µM. Several samples of skin from several scleroderma patients were similarly assayed and had the following level of 3DG: 15 µM, 130 µM, and 3.5 µM. Accordingly, these data demonstrate that the level of 3DG in the skin of scleroderma patients is significantly elevated compared with the level of 3DG in the skin of normal humans.

Example 26

Formulation of a liposome cream delivery system.

23.9 grams of BioCreme Concentrate from BioChemica International Inc. was blended with 2.9 grams cocoa butter, 1.4 grams shea butter, 2.2 grams aloe oil, 1.1 grams vitamin E, 3.7 grams glycerol, 51 grams water, 1.1 grams dimethicone and 10.8 grams Natipide II containing 1 gram arginine-HCl and 1 gram meglumine-HCl.

Example 27

Treatment of Psoriasis

A blinded study was conducted with 9 adult volunteers having 2-10% of their body surface area affected with psoriasis. Between 2 and 4 psoriasis-affected sites for each volunteer were chosen for treatment; only one type of cream was used on each volunteer. The volunteers were divided into 3 groups of 3 volunteers each, and the affected sites on the volunteers in each group were treated with twice daily applications of one of the following creams: 1) A base cream containing salicylic acid (1.9 %) ("Cream SA"); 2) A base cream containing salicylic acid (1.9 %) and
meglumine (5.5%) and arginine (3.8%) ("Cream SAMA"); or 3) A base cream containing meglumine (5.5%) and arginine (3.8%) ("Cream MA")

An expert grader was used to examine the skin areas. Assessments were made at the beginning of the study and after 3 weeks with respect to:

A. Erythema (0=no redness, 1=faint redness, 2=red coloration, 3=very bright red coloration, 4= deep red coloration);

B. Dryness (0=no dryness/scaling, 1=fine scale partially covering lesions, 2=fine to coarse scale covering most or all of the lesions, 3=coarse, non-tenacious scale predominates covering most or all of the lesions, 4=coarse, thick, tenacious scale over most or all lesions, rough surface);

C. Induration (0=no evidence of plaque elevation, 1=slight but definite plaque elevation, typically edges indistinct or sloped, 2=moderate plaque elevation with rough or sloped edges, 3=marked plaque elevation typically with hard or sharp edges, 4=very marked plaque elevation typically with hard sharp edges); and

D. Pruritis (0=no itching, 1=slightly bothersome itching, 2=bothersome itching, but no loss of sleep, 3=constant itching causing intense discomfort and loss of sleep).

The mean values for the expert grader’s scores at 0 weeks (beginning of study) and after 3 weeks are shown in Table F. A statistical t-test was used to determine the significance of any difference between the means. Bold values indicate p<0.05. The volunteers treated with the Cream SA exhibited a statistical improvement with respect to erythema, but no statistical improvement with respect to dryness, induration, or pruritis. The volunteers treated with the Cream SAMA exhibited a statistical benefit for erythema, induration and pruritis, and approached significance for dryness. The volunteers treated with the Cream MA exhibited a statistical benefit for dryness, induration, and purities, and exhibited a non-statistical improvement erythema. Cream MA exhibits clear benefits over Cream SA with respect to dryness, induration and pruritis. Cream SAMA provides clear benefits over Cream SA with respect to dryness, induration and pruritis.

Table F: Results of Psoriasis study over 3-week time period.
A 250 g male Sprague-Dawley rat was sacrificed with an overdose of pentobarbital and the pancreas removed and snap frozen in liquid nitrogen. The pancreas was pulverized in liquid nitrogen with 5 µmol of phenylphosphonic acid (an internal standard for quantitation) and six volumes of 5% perchloric acid containing 10 mmol/1 trans-L,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid. The resultant slurry was centrifuged at 8,000g at 4°C for 10 min. The supernatant was neutralized with KOH and was centrifuged again to remove the precipitate of potassium perchlorate. The supernatant was lyophilized to a powder and reconstituted in 1-ml of D_2O at pH 7.5 for NMR measurement. 31P-NMR spectra were obtained in a 10-mm probe at 161.98 MHz on a Bruker AM 400 spectrometer using 60° pulses and a 1.5 second repetition time. The spectra were acquired in blocks of 20,000 scans and were referenced to glycerophosphocholine set at 0.49 ppm. Quantitation of the FL3P resonance was determined by integration of peak area, setting the phenylphosphonic acid area equal to 5 umol. FL3P resonates at 6.23 ppm and was identified by spiking with authentic material as well as reduction with sodium borohydride to sorbitolysine 3-phosphate (5.95 ppm) and mannotolysine 3-phosphate (5.85 ppm). The concentration of FL3P in the pancreas was 28 µM.

The therapeutic creams set forth in Experimental Examples 29-36 contained 3-5.5% meglumine and 3-4% arginine as the active ingredients.

**Example 29**

**Psoriasis.**
Five adults with psoriasis applied a base cream containing meglumine and arginine and experienced decreased inflammation and dryness.

Example 30

Eczema

A seven year old girl with eczema used a base cream containing meglumine and arginine and experienced decreased inflammation, itch and dryness.

Example 31

Arthritis

Two female adults with arthritis used daily application of a base cream containing meglumine and arginine and experienced relief from joint pain, swelling and tenderness.

Example 32

Sinus headache

An adult male and female with headaches centered around the facial and forehead areas applied a base cream containing meglumine and arginine to the affected areas. Both experienced pain relief approximately 30 minutes after application.

Example 33

Acne

An adult woman with facial acne applied a base cream containing meglumine and arginine to affected skin areas and experienced a decrease in number/severity of lesions, and increased skin smoothness and softness.

Example 34

Razor burn

Two adult males with facial razor burn applied a base cream containing meglumine and arginine immediately after shaving and experienced a decrease in skin redness.
Example 35
Polycythemia

A female adult with skin rash due to polycythemia used a base cream supplemented with meglumine and arginine and experienced decreased inflammation and itching.

Example 36
Sodium Lauryl Sulfate Skin Irritation Trial

A clinical study was performed to determine the effectiveness of a base cream and a base cream containing meglumine and arginine to reduce redness (inflammation) and repair damage to the skin using a sodium lauryl sulfate (SLS) wound healing (irritation amelioration) test. The protocol included self assessments of the study participants, expert grader assessments and instrument measurements of evaporative water loss and redness. This was a single blind, controlled, randomized study.

The volar forearms of a group of twelve women volunteers from 18-55 years old were exposed to an irritant solution (0.3 ml of a 0.5% sodium lauryl sulfate solution) at six sites (three sites on each arm) for 18-24 hours. The four sites that were the most irritated were selected for further treatment with a twice-daily application of either a base cream (Product A) or a cream containing 3% meglumine and 3% arginine (Product B) for 7 days. The remaining two sites were not treated. At 1, 2, 3, 4, 7 and 8 days after the SLS application, the skin areas were assessed using a Minolta Chromameter (to measure color intensity), an expert grader (using an 8-point scale), and a DermaLab Modular System with TEWL Probe (to measure water loss).

On the eighth day of treatment, the participants filled out a self-assessment questionnaire. Responses are set forth in Table G.

<table>
<thead>
<tr>
<th>Feature of cream</th>
<th>Counts</th>
<th>Significance test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Product A</td>
<td>Product B</td>
</tr>
<tr>
<td>Quickest healing</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Least irritating</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Reduced redness</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Best feeling</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>
Example 37: Cloning and purification of recombinant F3K

The mouse cDNA for fructosamine-3-kinase (F3K) was obtained by reverse transcriptase-PCR (RT-PCR) using message RNA from mouse kidney (Ambion, Austin, TX) and cloned in frame into the baculovirus expression vector pFastBac version "b" (Invitrogen, Carlsbad, CA). The pFastBac vector contains a six-histidine amino acid tag ("6xHis"), which can be used for purification. The cloned F3K insert was sequenced and transferred into baculovirus for subsequent infection of Spodopiera frugiperda Sf9 cells. Cells were infected with recombinant baculovirus and harvested after 48 hours.

F3K was purified using a 6xHis fusion kit (Pierce, Rockford, IL). Briefly, the pellet from 50 ml volume of culture of infected cells (at a density of approximately 2 x 10^6 cells/ml) was resuspended using 1.5 ml of B-per lysis solution (Pierce) and gently rocked for 10 minutes. The suspension was transferred into a 2 ml microfuge tube and centrifuged at 27,000 x g for 10 minutes. The supernatant was then applied to a 1 ml nickel-based affinity chromatography column, washed according to the manufacturer's instructions, and protein was subsequently eluted two washes of 3 ml, using the manufacturer's elution buffer as provided. Fifty microliters of each fraction was tested for presence of enzyme activity. The eluted fractions containing F3K enzyme were then dialyzed overnight at 4°C against a dialysis buffer containing 10 mM HEPES, pH 7.0, 25 mM KCl, 0.1 mM EDTA, 10% glycerol, 100 µM PMSF, and 100 µM DTT. Protein purity was assessed by SDS-PAGE.

Example 38: Phosphate release assay (PRA)

Fructosamine-3-kinase (F3K) activity was measured in an assay that quantifies inorganic phosphate. F3K phosphorylates fructoselysine to produce fructoselysine-3-phosphate (FL3P). This product molecule undergoes a chemical rearrangement to produce fructose, 3-deoxyglucosone and inorganic phosphate.
An ammonium molybdate solution was used, containing 3.125 g of ammonium molybdate tetrahydrate dissolved in 200 ml of water. This solution was added to 500 ml of 7 N sulfuric acid and the volume adjusted up to 1000 ml using water. A stannous chloride solution was used, the solution containing 200 mg of stannous chloride dissolved in 30 ml of 0.5 N HCl.

The assay was performed in a 96-well clear plastic polystyrene plate. Each well contained 100 µl of 50 mM glycine, pH 9.8, 1 mM Mg-ATP, 1 mM fructoselysine, and F3K (i.e., sufficient enzyme to produce 0.5 nmols FL3P/min). The plate was incubated at 37°C for 1 hour and the reaction then quenched with 10 µl of 50 mM EDTA. The plate was kept at 20°C for 18 hours (to allow FL3P to decompose) and the levels of phosphate determined by adding 100 µl of ammonium molybdate solution and then 40 µl of stannous chloride solution. The color development was complete within 5 minutes and was stable for at least 45 minutes. The optical density (O.D.) was read in a spectrophotometer at 630 nm.

Optical blank reaction mixtures (i.e., reaction mixtures without fructoselysine substrate) were subtracted from each reaction. Under these standard conditions, a ΔOD$_{630}$ of 0.5 units was obtained. This corresponded to 22.5 nmols of phosphate, as compared to a theoretical yield of 30 nmols (i.e., 0.5 nmols/min for 60 min). This calculation identified the half-life of FL3P to be approximately 9 hours. A standard curve for phosphate was included on each plate as an additional control (see Figure 19). GC-MS measurement of 3DG from identical incubations gave results of 21.8 ± 0.3 nmols 3DG (n=3). The molar amount of 3DG produced should be equal to the moles of phosphate produced; thus, this independent measurement of F3K activity was in excellent agreement with the phosphate value.

ATP (Sigma, ultrapure) was very stable under the conditions used for the reaction. One millimolar Mg-ATP incubated under the standard reaction conditions with F3K and without fructoselysine substrate results in an OD value between 0.12 and 0.14 OD units using the phosphate color reaction. This result serves as the "blank" value.

Figure 19 illustrates the standard curve obtained with phosphate. This reaction is linear with respect to enzyme. The standard reaction was run with three different concentrations of enzyme, 0.5-, 1-, and 2-times the normal assay amounts of
enzyme. The data in Figure 20 demonstrates a linear relationship between increased production of phosphate and enzyme concentration.

Example 39: Screening of Inhibitors for F3K Activity

A number of compounds were screened for inhibition of F3K activity. Compounds illustrated in Figures 21A-21G were used in the phosphate assay as described above, and were purchased from ChemBiidge (San Diego, CA), dissolved in DMSO, and used in the assay at a final concentration of 100 µM. Table H illustrates the inhibitory activity of compounds illustrated in Figures 21A-21G. Inhibitory activity is given as percent inhibition in the presence of 100 µM compound. Each value is the average of three separate repetitions of the experiment.

Table H: F3K inhibitory activity of various compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 21A (furan)</td>
<td>65%</td>
</tr>
<tr>
<td>Figure 21B</td>
<td>19%</td>
</tr>
<tr>
<td>Figure 21C (thiazolidinedione)</td>
<td>34%</td>
</tr>
<tr>
<td>Figure 21D (pyramidyl)</td>
<td>17%</td>
</tr>
<tr>
<td>Figure 21E</td>
<td>65%</td>
</tr>
<tr>
<td>Figure 21F</td>
<td>46%</td>
</tr>
<tr>
<td>Figure 21G (pyramidyl)</td>
<td>23%</td>
</tr>
</tbody>
</table>

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.
What is Claimed:

1. A method of inhibiting fructosamine-3-kinase (F3K) activity in the skin of a mammal, said method comprising administering to said mammal an effective amount of an inhibitor of F3K activity, wherein the inhibitor of F3K activity is not meglumine.

2. The method of claim 1, wherein said inhibitor is administered via a route selected from the group consisting of topical, oral, rectal, vaginal, intramuscular, and intravenous.

3. The method of claim 2, wherein said inhibitor is administered via a topical route.

4. The method of claim 1, wherein said inhibitor of fructosamine-3-kinase activity comprises a compound of formula VIII:

   ![Formula VIII](image)

   wherein

   \( G^{10} \) is independently selected at each occurrence from the group consisting of formulae VIII₁, VIII₂, and VIII₃, VIII₄, and VIII₅.
R³ is independently selected at each occurrence from the group consisting of
Hydrogen, -OH, -CH₂OH, -CH₃, and G¹ provided that G¹ may be selected no
more than once for each occurrence of VIII¹, VIII², VIII³, VIII⁴, or VIII⁵;
G¹ is independently selected at each occurrence from the group consisting of
formulae VIII⁶, VIII⁷, VIII⁸, VIII⁹, and VIII¹⁰:

R⁴ is independently selected at each occurrence from the group consisting of
Hydrogen, -OH, -CH₂OH, and -CH₃.

5. The method of claim 4, wherein said compound is:

or a pharmaceutically acceptable salt thereof.

6. The method of claim I₃ wherein said inhibitor of fructosamine-3-
kinase activity comprises a compound of formula X:

wherein
R⁵ is independently selected at each occurrence from the group consisting of Hydrogen; F; Cl; Br; I; (Ci-C₆)alkyl; (Ci-C₆)alkenyl; (Ci-C₆)alkoxy; OH; NO₂; O=N; C(=O)(C₃-C₆)alkyl; (C₂-C₆)alkyl-OR₂; phosphonato; NR₂; NHC(=O)(C₃-C₆)alkyl; sulfamyl; carbamyl; OC(=O)(C₃-C₆)alkyl; O(C₂-C₆)alkylene-N((C₁-C₆)alkyl)₂; and (Ci-C₆)perfluoroalkyl; or a stereoisomer or pharmaceutically acceptable salt of such a compound.

R² independently selected at each occurrence from the group consisting of hydrogen and (Ci-C₆)alkyl;

Ar is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; (Ci-C₆)alkyl; (Ci-C₆)alkenyl; (Ci-C₆)alkoxy; OH; NO₂; C≡N; C(=O)(Ci-C₆)alkyl; (C₂-C₆)alkylene-OR₂; phosphonato; NR₂; NHC(=O)(Ci-C₆)alkyl; sulfamyl; carbamyl; OC(=O)(Ci-C₆)alkyl; O(C₂-C₆)alkylene-N((Ci-C₆)alkyl)₂; and (Ci-C₆)perfluoroalkyl; or a stereoisomer or pharmaceutically acceptable salt therof.

7. The method of claim 6, wherein said compound is:

![Chemical Structure](image)

or a pharmaceutically acceptable salt thereof.

8. The method of claim 1, wherein said inhibitor of fructosamine-3-kinase activity comprises a compound of formula IX:

![Chemical Structure](image)

wherein

R⁵ is independently selected at each occurrence from the group consisting of Hydrogen; F; Cl; Br; I; (Ci-C₆)alkyl; (Ci-C₆)alkenyl; (d-C₆)alkoxy; OH;
NO₂; C≡N; C(=O)(C₃ alkyl); (C₂-C₆ alkenyl-OR₂; phosphonato; NR₂; NHC(=O)(C₃ alkyl), sulfamyl; carbamyl; OC(=O)(C₃ alkyl); O(C₂-C₆ alkenyl-N((C₆ alkyl)₂; and (C₃ perfluoroalkyl; O(C₀-C₆ Alkyl)Ar;

R² independently selected at each occurrence from the group consisting of hydrogen and (C₁-C₆ alkyl);

Ar is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; (Cᵢ-C₆ alkyl); (Cᵢ-C₆ alkenyl; (Cᵢ-C₆ alkoxy; OH; NO₂; C≡N; C(=O)(C₃ alkyl); (C₂-C₆ alkenyl-OR₂; phosphonato; NR₂; NHC(=O)(Cᵢ-C₆ alkyl), sulfamyl; carbamyl; OC(=O)(Cᵢ-C₆ alkyl; O(C₂-C₆ alkenyl-N((Cᵢ-C₆ alkyl)₂; and (Cᵢ-C₆ perfluoroalkyl;

or a stereoisomer or pharmaceutically acceptable salt of such a compound.

9. The method of claim 1, wherein said compound is selected from the group consisting of:

\[ \text{IXA; } \]

\[ \text{IXB; } \]

\[ \text{IXC; } \]

\[ \text{IXD; } \]
10. The method of claim 1, wherein said mammal is a human.

11. The method of claim 1, wherein said inhibitor comprises from about 0.0001% to about 15% by weight of said pharmaceutical composition.

12. The method of claim 1, wherein said inhibitor is administered as a controlled-release formulation.

13. The method of claim 1, wherein said pharmaceutical composition is selected from the group consisting of a lotion, a cream, a gel, a liniment, an ointment, a paste, a solution, a powder, and a suspension.

14. The method of claim 13, wherein said composition further comprises a moisturizer, a humectant, a demulcent, oil, water, an emulsifier, a

or a pharmaceutically acceptable salt therof.
thickener, a thinner, a surface active agent, a fragrance, a preservative, an antioxidant, a hydro-tropic agent, a chelating agent, a vitamin, a mineral, a permeation enhancer, a cosmetic adjuvant, a bleaching agent, a depigmentation agent, a foaming agent, a conditioner, a viscosifier, a buffering agent, and a sunscreen.

15. The method of claim 1, wherein said compound inhibits advanced glycation end product modified protein formation.

16. The method of claim 1, wherein said compound inhibits a function selected from the group consisting of protein crosslinking, apoptosis, formation of reactive oxygen species, and mutagenesis.

17. The method of claim 1, wherein said compound stimulates 3DG detoxification.

18. The method of claim 1, wherein said compound stimulates 3DG clearance.

19. A method of treating an alpha-dicarbonyl sugar associated skin disease or disorder in a mammal, said method comprising, administering to said mammal an alpha-dicarbonyl sugar inhibiting amount of a compound which inhibits F3K activity, thereby treating an alpha-dicarbonyl sugar associated skin disease or disorder of a mammal, wherein the inhibitor of F3K activity is not meglumine.

20. The method of claim 19, wherein said alpha-dicarbonyl sugar associated skin disease or disorder comprises a disease or disorder associated with a function selected from the group consisting of protein crosslinking, apoptosis, mutagenesis, and formation of reactive oxygen species.

21. The method of claim 19, wherein said alpha-dicarbonyl sugar associated skin disease or disorder comprises a disease or disorder associated with advanced glycation end product modified protein formation.
22. The method of claim 19, wherein said disease or disorder is selected from the group consisting of skin cancer, psoriasis, skin aging, skin wrinkling, hyperkeratosis, hyperplasia, acanthosis, papillomatosis, dermatosis, rhinophyma, scleroderma, eczema, seborrhea, and rosacea.

23. The method of claim 22, wherein the compound is administered in combination with a topical steroid.

24. The method of claim 23, wherein the topical steroid is selected from the group consisting of hydrocortisone, clobetasone butyrate, triamcinolone acetonide, fluocinolone acetonide, betamethasone valerate, betamethasone dipropionate, diflucortolone valerate, fluticasone valerate, hydrocortisone 17-butyrate, mometasone furoate, methylprednisolone aceponate, betamethasone dipropionate, and clobetasol propionate.

25. The method of claim 19, wherein said alpha-dicarbonyl sugar associated skin disease or disorder comprises a disease or disorder associated with acne.

26. The method of claim 24, wherein the compound is administered in combination with at least one additional composition for treating acne.

27. The method of claim 26, wherein the additional composition comprises at least one of the members selected from the group consisting of benzoyl peroxide, salicylic acid and erythromycin.

28. A kit for administering a compound which inhibits F3K activity in the skin of a mammal, said kit comprising a compound which inhibits F3K activity, a standard, an applicator, and an instructional material for the use thereof, wherein the inhibitor of F3K activity is not meglumine.

29. The kit of claim 28, wherein said mammal is a human.
30. A method of treating a disease associated with the presence of 3DG in a mammal, said method comprising administering to said mammal a composition comprising an F3K inhibitor, wherein the F3K inhibitor is not meglumine.

31. A method of treating an inflammatory condition in a mammal, the method comprising administering to the mammal a composition comprising an F3K inhibitor, the administration resulting in reduction or elimination of the alpha-dicarbonyl sugar at a site in the mammal, said site being affected by the inflammatory condition, thereby treating the inflammatory condition, wherein the F3K inhibitor is not meglumine.

32. A method of treating pain in a mammal, the method comprising administering to the mammal a composition comprising an F3K inhibitor, the administration resulting in reduction or elimination of the alpha-dicarbonyl sugar at a site in the mammal, said site being affected by the pain, thereby treating the pain, wherein the F3K inhibitor is not meglumine.

33. A method of treating itch in a mammal, the method comprising administering to the mammal a composition comprising an F3K inhibitor, the administration resulting in reduction or elimination of the alpha-dicarbonyl sugar at a site in the mammal, said site being affected by the itch, thereby treating the itch, wherein the F3K inhibitor is not meglumine.

34. The method of claim 31, wherein the inflammatory condition is selected from the group consisting of allergic conditions, Alzheimer's disease, anemia, angiogenesis, aortic valve stenosis, atherosclerosis, thrombosis, rheumatoid arthritis, osteoarthritis, gout, gouty arthritis, acute pseudogout, acute gouty arthritis, inflammation associated with cancer, congestive heart failure, cystitis, fibromyalgia, fibrosis, glomerulonephritis, inflammation associated with gastro-intestinal disease, inflammatory bowel diseases, irritable bowel diseases, kidney failure, glomerulonephritis, myocardial infarction, ocular diseases, pancreatitis, psoriasis, reperfusion injury or damage, respiratory disorders, restenosis, septic shock, endotoxic shock, urosepsis, stroke, surgical complications, systemic lupus.
erthymotosus, transplantation associated arteriopathy, graft vs. host reaction, allograft rejection, chronic transplant rejection, vasculitis, and specifics relating to the condition, where it might arise and how the composition might be administered.

35. The method of claim 34, wherein the composition further comprises at least one of the members selected from the group consisting of an antacid, a probiotic agent, an H-2 blockers, and a proton pump inhibitor.

36. The method of claim 32, wherein the pain is selected from the group consisting of arachnoiditis, arthritis, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, gout, tendonitis, bursitis, sciatica, spondylolisthesis, radiculopathy, bum pain, cancer pain, headaches, migraines, cluster headaches, tension headaches, trigeminal neuralgia, myofascial pain, neuropathic pain, pain associated with diabetic neuropathy, reflex sympathetic dystrophy syndrome, phantom limb pain, post-amputation pain, tendonitis, tenosynovitis, postherpetic neuralgia, shingles-associated pain, central pain syndrome, trauma-associated pain, vasculitis, pain associated with infections, skin tumors, cysts, pain associated with tumors associated with neurofibromatosis, pain associated with strains, bruises, dislocations, fractures, and pain due to exposure to chemicals.

37. The method of claim 33, wherein the itch is the result of a condition selected from the group consisting of cutaneous itch, neuropathic itch, neurogenic itch, mixed-type itch, and psychogenic itch.

38. The method of claim 36, wherein the cancer is selected from the group consisting of NSCLC, ovarian cancer, pancreatic cancer, breast carcinoma, colon carcinoma, rectum carcinoma, lung carcinoma, oropharynx carcinoma, hypopharynx carcinoma, esophagus carcinoma, stomach carcinoma, pancreas carcinoma, liver carcinoma, gallbladder carcinoma, bile duct carcinoma, small intestine carcinoma, urinary tract carcinoma, kidney carcinoma, bladder carcinoma, urothelium carcinoma, female genital tract carcinoma, cervix carcinoma, uterus carcinoma, ovarian carcinoma, choriocarcinoma, gestational trophoblastic disease, male genital tract carcinoma, prostate carcinoma, seminal vesicles carcinoma, testes
carcinoma, germ cell tumors, endocrine gland carcinoma, thyroid carcinoma, adrenal
carcinoma, pituitary gland carcinoma, skin carcinoma, hemangiomas, melanomas,
sarcomas, bone and soft tissue sarcoma, Kaposi's sarcoma, tumors of the brain, tumors
of the nerves, tumors of the eyes, tumors of the meninges, astrocytomas, gliomas,
glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas,
meningiomas, solid tumors arising from hematopoietic malignancies, and solid
tumors arising from lymphomas.

39. The method of claim 38, wherein the solid tumors arising from
hematopoietic malignancies is selected from the group consisting of leukemias,
chloromas, plasmacytomas and the plaques and tumors of mycosis fungoides and
cutaneous T-cell lymphoma/leukemia.

40. The method of claim 34, wherein the gastro-intestinal disease is
selected from the group consisting of aphthous ulcers, pharyngitis, esophagitis, peptic
ulcers, gingivitis, periodontitis, oral mucositis, gastrointestinal mucositis, nasal
mucositis, irritable bowel disease and proctitis.

41. The method of claim 34, wherein the inflammatory bowel disease
is selected from the group consisting of Crohn's disease, ulcerative colitis,
indeterminate colitis, necrotizing enterocolitis, pouchitis and infectious colitis.

42. The method of claim 34, wherein the ocular disease is selected
from the group consisting of conjunctivitis, retinitis, and uveitis.

43. The method of claim 34, wherein the respiratory disorder is
selected from the group consisting of asthma, mononuclear-phagocyte dependent lung
injury, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, adult
respiratory distress syndrome, acute chest syndrome in sickle cell disease, cystic
fibrosis.

44. The method of claim 1, wherein said composition further
comprises a non-steroidal anti inflammatory drug (NSAID).
45. The method of claim 44, wherein said non-steroidal anti-inflammatory drug (NSAID) is selected from the group consisting of ibuprofen (2-(isobutylphenyl)-propionic acid); methotrexate (N-[4-(2,4 diamino 6-pteridinylmethyl]methylene]benzoyl-L-glutamic acid); aspirin (acetylsalicylic acid); salicylic acid; diphenhydramine (2-(diphenylmethoxy)-NN-dimethylethylamine hydrochloride); naproxen (2-naphthaleneacetic acid, 6-methoxy-9-methyl-, sodium salt, (-)); phenylbutazone (4-butyl-1,2-diphenyl-3,5-pyrazolidinedione); sulindac-(2)-5-fluoro-2-methyl-1-[[p-(methylsulfinyl)phenyl]methylene]-1H-indene-3-acetic acid; diflunisal (2',4',-difluoro-4-hydroxy-3-biphenylcarboxylic acid; piroxicam (4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-2-carboxamide 1,1-dioxide, an oxicam; indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-H-indole-3-acetic acid); meclofenamate sodium (N-(2,6-dichloro-m-tolyl) anthranilic acid, sodium salt, monohydrate); ketoprofen (2-(3-benzoylphenyl)-propionic acid; tolmetin sodium (sodium l-methyl-5-(4-methylbenzoyl-1H-pyrrole-2-acetate dihydrate); diclofenac sodium (2-[(2,6-dichlorophenyl)amino]benzeneacetic acid, monosodium salt); hydroxychloroquine sulphate (2-[[4-[(7-chloro-4-quinolyl)amino]pentyl]ethylamino]ethanol sulfate (1:1); penicillamine (3-mercaptop-D-valine); flurbiprofen ([l,l-biphenyl]-4-acetic acid, 2-fluoro-alphamethyl-, (+,-)); cetodolac (1-8-diethyl-13,4,9, tetra hydropyrano-[3-4-13]indole-l-acetic acid; mefenamic acid (N-(2,3-xylyl)anthranilic acid; and diphenhydramine hydrochloride (2-diphenyl methoxy-N,N-di-methylethylamine hydrochloride).

46. The method of claim 1, wherein the composition further comprises arginine.

47. A method of preventing the formation of 3DG in a mammal, said method comprising administering to said mammal an F3K inhibitor, wherein the F3K inhibitor is not meglumine.

48. The method of claim 1, wherein said inhibitor of fructosamine-3-kinase activity comprises a compound of formula I:
wherein:

A \( r \) is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; \((C_1-C_6)\)alkyl; \((C_1-C_6)\)alkenyl; \((C_i-C_6)\)alkoxy; \(\text{OH; NO}_2; \text{C} \equiv \text{N; C(=0)(C}_3-C_3)\)alkyl; \((C_2-C_4)\)alkylene-\(\text{OR}_2\); phosphonato; \(\text{NR}_2^2; \text{NHC(O)(C}_3-C_3)\)alkyl; sulfamyl; carbamyl; \(\text{OC(O)(C}_3-C_3)\)alkyl; \(\text{O(C}_2-C_6)\)alkylene-\(\text{N((C}_3-C_3)\)alkyl\)2; and \((\text{C}_2-C_3)\)perfluoroalkyl; and

\(R^2\) is independently selected at each occurrence from the group consisting of hydrogen and \((\text{C}_i-C_6)\)alkyl or a stereoisomer or pharmaceutically acceptable salt of such a compound.

49. The method of claim 48, wherein said compound is:

![](image)

or a pharmaceutically acceptable salt thereof.

50. The method of claim 1, wherein said inhibitor of fructosamine-3-kinase activity comprises a compound of formula II:

![](image)
wherein

\( \text{Ar} \) is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; \((\text{Ci-C}_6)\text{alkyl}\); \((\text{Ci-C}_6)\text{alkenyl}\); \((\text{Ci-C}_6)\text{alkoxy}\); \text{OH}; \text{NO}_2; \text{C}=\text{N}; \text{C}(=\text{O})(\text{Ci-C}_3)\text{alkyl}; \text{(C}_2\text{-C}_6)\text{alkylene-OR}^2; \text{phosphonato}; \text{NR}^2_2; \text{NHC}(=\text{O})(\text{C}_1\text{-C}_6)\text{alkyl}; \text{sulfamyl}; \text{carbamyl}; \text{OC}(=\text{O})(\text{C}_1\text{-C}_3)\text{alkyl}; \text{O(C}_2\text{-C}_6)\text{alkylene-N((C}_1\text{-C}_6)\text{alkyl})}_2; \text{and (C}_2\text{-C}_6)\text{perfluoroalkyl}; \)  

\( \text{G}^1 \) is independently selected at each occurrence from the group consisting of \text{C}=\text{O} and \text{CH}_2, provided that at least one occurrence of \( \text{G}^1 \) is \text{C}=\text{O};  

\( -\text{L}- \) is selected from the group consisting of \(-\text{NH-C}(=\text{O})\)-, \(-\text{C}(=\text{O})\text{-NH}-\), \(-\text{O}-\), \(-\text{S}-\), and \(-\text{NR}^2\)-;  

\( \text{R}^1 \) independently selected at each occurrence from the group consisting of hydrogen; halogen; \((\text{d-C}_6)\text{alkyl}\); \((\text{Ci-C}_6)\text{alkenyl}\); \((\text{Ci-C}_6)\text{alkoxy}\); \text{OH}; \text{NO}_2; \text{C}=\text{N}; \text{C}(=\text{O})(\text{Ci-C}_3)\text{alkyl}; \text{(C}_2\text{-C}_6)\text{alkylene-OR}^2; \text{phosphonato}; \text{NR}^2_2; \text{NHC}(=\text{O})(\text{Ci-C}_3)\text{alkyl}; \text{sulfamyl}; \text{carbamyl}; \text{OC}(=\text{O})(\text{Ci-C}_3)\text{alkyl}; \text{O(C}_2\text{-C}_6)\text{alkylene-N((Ci-C}_3)\text{alkyl})}_2; \text{and (C}_2\text{-C}_6)\text{perfluoroalkyl}; \) and  

\( \text{R}^2 \) is independently selected at each occurrence from the group consisting of hydrogen and \((\text{Ci-C}_6)\text{alkyl}; \) or a stereoisomer or pharmaceutically acceptable salt of such a compound.

51. The method of claim 50, wherein said compound is:

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\text{\includegraphics[width=0.5\textwidth]{image.png}}
```

or a pharmaceutically acceptable salt therof.
52. The method of claim 1, wherein said inhibitor of fructosamine-3-kinase activity comprises a compound of formula III:

\[ \text{III} \]

wherein

- \( G^2 \) is selected from the group consisting of formulae III \(^1\), III \(^2\), and III \(^3\):

\[ \text{in}^1 \quad \text{in}^2 \quad \text{in}^3 \]

- \( G^3 \) is selected from the group consisting of \( \text{NR}^2 \), \( \text{C(R}^2\text{)}_2 \), \( \text{O} \), and \( \text{S} \);

- \( G^4 \) is \( \text{C(R}^2\text{)}_2 \); and

- \( \text{Ar} \) is independently selected from each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; \( \text{(Ci-Cs)} \) alkyl; \( \text{(Ci-C}_6\text{)} \) alkenyl; \( \text{C}_r\text{C}_6 \) alkoxy; \( \text{OH} \); \( \text{NO}_2 \); \( \text{C} \equiv \text{N} \); \( \text{C(=O)} \) \( \text{O(C}_3\text{)} \) alkyl; \( \text{(C}_2\text{C}_r \) alkyne-OR \(^2\); phosphonato; \( \text{NR}^2 \); \( \text{NHC(=O)} \) \( \text{C}_6\text{)} \) alkyl; \( \text{sulfamyl} \); carbamyl; \( \text{OC(=O)} \) \( \text{C}_3\text{)} \) alkyl; \( \text{O(C}_2\text{C}_6 \) alkyne-N(\( \text{C}_3\text{)} \) alkyl) \(^2\); and \( \text{(Ci-C}_3 \) perfluoroalkyl;

- \( \text{R}^2 \) independently selected from each occurrence from the group consisting of hydrogen and \( \text{(Ci-C6)} \) alkyl;

- \( m \) is 2 or 3; and

- \( n \) is 1, 2, or 3;

or a stereoisomer or pharmaceutically acceptable salt of such a compound.
53. The method of claim 52, wherein said compound is:

![Chemical Structure](image)

or a pharmaceutically acceptable salt therof.

54. The method of claim 1, wherein said inhibitor of fructosamine-3-kinase activity comprises a compound of formula IV:

![Chemical Structure](image)

wherein

- $G^3$ is selected from the group consisting of $NR^2$, $C(R^2)_{2}$, $O$, and $S$;
- $G^5$ is independently selected at each occurrence from the group consisting of $NR^2$, $O$, and $S$;
- $G^6$ is selected from the group consisting of $Ar$, $Ar-((C_1-C_6)alkylene)$, and formula IV$^1$:

![Chemical Structure](image)

- $G^4$ is $C(R^2)_{2}$;
- $G^7$ is selected from the group consisting of $Ar$ and $Ar-((C_1-C_6)alkylene)$;
- $Ar$ is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with
one or more substituents, independently selected from halogen; (C₁₋₆)alkyl; (C₁₋₆)alkenyl; (d-C₆)alkoxy; OH; NO₂; C=N; C(=O)(C₁₋₃)alkyl; (C₂₋₆)alkylene-OR²; phosphonato; NR²₂; NH(O)(C₁₋₆)alkyl; sulfamyl; carbamyl; OC(=O)(C₁₋₆)alkyl; O(C₂₋₆)alkylene-N((C₁₋₆)alkyl)₂; and (C₁₋₆)perfluoroalkyl; and

R² independently selected at each occurrence from the group consisting of hydrogen and (C₁₋₆)alkyl;
or a stereoisomer or pharmaceutically acceptable salt of such a compound.

55. The method of claim 54, wherein said compound is:

![Chemical Structure](image)

or a pharmaceutically acceptable salt thereof.

56. The method of claim 1, wherein said inhibitor of fructosamine-3-kinase activity comprises a compound of formula V:

![Chemical Structure](image)

V

wherein
G^3 is selected from the group consisting of NR^2, C(R^2)\_2, O, and S;

G^5 is independently selected at each occurrence from the group consisting of NR^2, O, and S;

G^7 is independently selected at each occurrence from the group consisting of Ar and Ax-(Ci-C\_6)alkylene);

Ar is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; (Ci-C\_6)alkyl; (Ci-C\_6)alkenyl; (C\_2-C\_6)alkoxy; OH; NO\_2; C≡N; C(=O)O(Ci-C\_6)alkyl; (C\_2-C\_6)alkylene-OR^2; phosphonato; NR^2\_2; NHC(=O)(Ci-C\_6)alkyl; sulfamyl; carbamyl; OC(=O)(C\_2-C\_6)alkyl; O(C\_2-C\_6)alkylene-N((C\_6-C\_6)alkyl)\_2; and (Ci-C\_6)perfluoroalkyl; and

R^2 independently selected at each occurrence from the group consisting of hydrogen and (Ci-C\_6)alkyl;
or a stereoisomer or pharmaceutically acceptable salt of such a compound.

57. The method of claim 56, wherein said compound is:

![Chemical Structure](image)

or a pharmaceutically acceptable salt therof.

58. The method of claim 1, wherein said inhibitor of fructosamine-3-kinase comprises a compound of formula VI:
wherein

$G^5$ is selected from the group consisting of NR$^2$, O$_5$ and S;

$G^6$ is selected from the group consisting of Ar, Ar-((Ci-C$_6$)alkylene) and formula V:

$$\begin{align*}
V^1
\end{align*}$$

$G^4$ is C(R$_2$)$_2$;

$G^7$ is selected from the group consisting of Ar and Ar-((Ci-C$_6$)alkylene);

$G^8$ is N or CR$_\text{I}$;

Ar is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; (Ci-C$_6$)alkyl; (Ci-C$_6$)alkenyl; (C$_r$C$_6$)alkoxy; OH; NO$_2$; C≡N; C(=O)O(Ci-C$_3$)alkyl; (C$_2$-C$_6$)alkylene-OR; phosphonato; NR$^2$; NHC(=O)(Ci-C$_6$)alkyl; sulfamyl; carbamyl; OC(=O)(C$_r$C$_6$)alkyl; and (Ci-C$_3$)perfluoroalkyl;

R$_1$ independently selected at each occurrence from the group consisting of hydrogen; halogen; (Ci-C$_6$)alkyl; (C$_r$C$_6$)alkenyl; (C$_2$-C$_6$)alkoxy; OH; NO$_2$; C≡N; C(=O)O(Ci-C$_3$)alkyl; (C$_2$-C$_6$)alkylene-OR; phosphonato; NR$^2$; NHC(=O)(Ci-C$_3$)alkyl; sulfamyl; carbamyl; OC(=O)(Ci-C$_3$)alkyl; O(C$_2$-C$_6$)alkylene-N((Ci-C$_6$)alkyl)$_2$; and (Ci-C$_3$)perfluoroalkyl; and

R$^2$ independently selected at each occurrence from the group consisting of hydrogen and (Ci-C$_6$)alkyl;
or a stereoisomer or pharmaceutically acceptable salt of such a compound.

59. The method of claim 58, wherein said compound is:

\[
\text{VII}
\]

or a pharmaceutically acceptable salt thereof.

60. The method of claim 1, wherein said inhibitor of fructosamine-3-kinase activity comprises a compound of formula VII:

wherein:

- \( G^8 \) is \( N \) or \( CR^1 \);
- \( G^9 \) is \( O \) or \( S \);
- \( Ar \) is independently selected at each occurrence from the group consisting of aryl and heteroaryl, any of said aryl or heteroaryl optionally substituted with one or more substituents, independently selected from halogen; \((C_1-C_6)alkyl\); \((C_1-C_6)alkenyl\); \((C_1-C_6)alkoxy\); \(OH\); \(NO_2\); \(C\equiv N\); \(C(=O)O(C_1-C_3)alkyl\); \((C_2-C_6)alkylene-OR^2\); phosphonato; \(NR^2\); \(NHC(=O)(C_1-C_6)alkyl\); sulfamyl; carbamyl; \(OC(K))(C_2-C_6)alkyl\); \(O(C_2-C_6)alkylene-N((C_1-C_3)alkyl)2\); and \((C_1-C_3)perfluoroalkyl\); and
R\(^1\) independently selected at each occurrence from the group consisting of hydrogen; halogen; (Ci-C\(_6\))alkyl; (Ci-C\(_6\))alkenyl; (Ci-C\(_6\))alkoxy; OH; NO\(_2\); C≡N; C(=O)(Ci-C\(_3\))alkyl; (C\(_2\)-C\(_6\))alkylene-OR\(_2\); phosphonato; NR\(_2\); NHC(=O)(Ci-C\(_6\))alkyl; sulfamyl; carbamyl; OC(=O)(Ci-C\(_3\))alkyl; O(C\(_2\)-C\(_6\))alkylene-N((C\(_1\)-C\(_6\))alkyl)\(_2\); and (Ci-C\(_3\))perfluoroalkyl; and

R\(^2\) independently selected at each occurrence from the group consisting of hydrogen and (Ci-C\(_6\))alkyl;

or a stereoisomer or pharmaceutically acceptable salt of such a compound.

61. The method of claim 60, wherein said compound is:

![Chemical Structure](image)

or a pharmaceutically acceptable salt thereof.
**FIG. 7A**

**Normoglycemics**

\[ y = 39.774x - 6.6853 \]

\[ R = 0.89 \]

**FIG. 7B**

**Diabetics**

\[ y = 14.605x + 60.739 \]

\[ R = 0.74 \]
FIG. 10

meqflr aerq tattafggp gacgisegra yddaqpvf kvnrtqaq mfgdovsdle
airst glvrv prpmkvidlp ggaaafmeh lkmksllaq skigomadl h lynlkhrek
ikeoentvrg regeqeqqyv dkkfnftvio ogfipqvnw qddwptfsh hriqalddil
ekdyanevar elwrdri ev pddfgleiv palhpdiw s gnaedvqpp liydpsfyyg
hsfsliai mfggfrapp tafrnkplp pgsdleilfylq dfmyihwhn hsfreyreps
lgttnrllk

FIG. 11
GLUCOSE + LYSINE

GLYCATED PROTEIN

Catabolism

FRUCTOSELYSINE

AMADORASE

DYN 12

FL3P

Non-Enzymatic Path to 3-DG

3DF

Natural detoxification by aldose reductase (AR)
(AR inhibited by 3DG)

3DG

ROS

AGEs

Lysine + DYN 100

PL

Aging
Alzheimer's
Apoptosis
Atherosclerosis
Cancer
Dementia
Diabetic Complications
Inflammation
Scleroderma

FIG. 16
Aging
Alzheimer's
Apoptosis
Atherosclerosis
Cancer
Dementia
Diabetic Complications
Inflammation
Scleroderma

ROS

AGES

3DG

FIG. 17
**Schiff base**

**3DG**

**crosslinked protein**

**non-crosslinked protein**

**Inhibition of crosslinking by nucleophilic agents such as GSH or penicillamine**
FIG. 19

y = 0.0203x + 0.0331, R^2 = 1

FIG. 20

hF3K concentration