

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
3 October 2002 (03.10.2002)

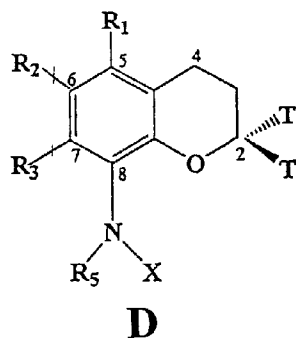
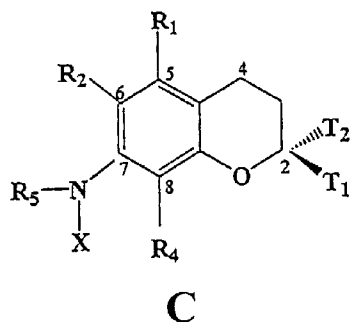
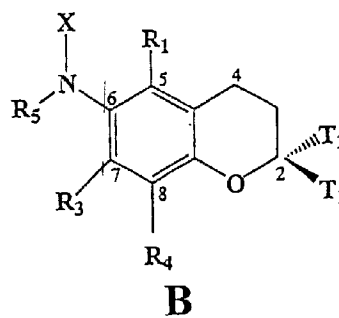
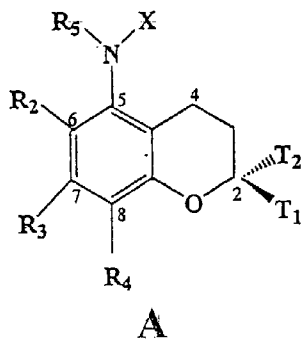
PCT

(10) International Publication Number
WO 02/076937 A2

- (51) International Patent Classification⁷: C07D
- (21) International Application Number: PCT/US02/11265
- (22) International Filing Date: 21 March 2002 (21.03.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/278,521 23 March 2001 (23.03.2001) US
- (71) Applicant (for all designated States except US): SONUS PHARMACEUTICALS, INC. [US/US]; 22026 20th Avenue, S.E., Bothell, WA 98021 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): LAMBERT, Karel, J. [US/US]; P.O. Box 971, Woodinville, WA 98071 (US). LAL, Manjari [IN/US]; 5001 - 148th Avenue N.E., Apt. D101, Bellevue, WA 98007 (US).
- (74) Agent: RENZONI, George, E.; Christensen, O'Connor, Johnson, Kindness PLLC, 1420 Fifth Avenue, Suite 2800, Seattle, WA 98101 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— without international search report and to be republished upon receipt of that report

[Continued on next page]

(54) Title: AMINOBENZOPYRAN DERIVATIVES AND COMPOSITIONS



(57) Abstract: Benzopyran derivatives including aminobenzopyran derivatives are described. Compositions that include the aminobenzopyran derivatives are also provided.



WO 02/076937 A2



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

AMINOBENZOPYRAN DERIVATIVES AND COMPOSITIONS

FIELD OF THE INVENTION

The present invention relates to aminobenzopyran derivatives and compositions
5 including the derivatives.

BACKGROUND OF THE INVENTION

Benzopyrans are an important class of molecules that includes vitamin E,
tocopherols, and tocotrienols. These compounds have been developed to improve the
water solubility of the oily vitamin so as to improve dietary or parenteral uptake of
10 vitamin E in certain clinical and veterinary conditions, to discover novel medicaments,
and to develop novel antioxidants. Only recently, the solubilization properties of
tocopherols, tocopherol acetate, tocopherol succinate, TPGS and tocotrienols have been
recognized. However, the chemistry of tocopherol derivatization for this purpose has not
advanced much since the introduction of TPGS in 1951, and has not encompassed use of
15 aminobenzopyrans as efficient starting materials for derivatization. Previous work has
not recognized the use of these derivatives as surfactants, or as pharmaceutical excipients
in emulsions, nanoemulsions, microemulsions, liposomes or micellar solutions.

The present invention seeks to fulfill this need and provides further related
advantages.

20 SUMMARY OF THE INVENTION

In one aspect the present invention provides benzopyran derivatives. In one
embodiment, the benzopyran derivatives are amine derivatives. In another embodiment,
the benzopyran derivatives are amide derivatives.

In another aspect of the invention, compositions that include the benzopyran
25 derivatives are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will
become more readily appreciated as the same become better understood by reference to
the following detailed description, when taken in conjunction with the accompanying
30 drawings, wherein:

FIGURES 1A-D are chemical structures of representative benzopyran amine
derivatives of the invention;

FIGURES 2A-D are chemical structures of representative benzopyran amide derivatives of the invention;

FIGURE 3 is the chemical structure of vitamin E;

FIGURE 4 is the chemical structure of d- α -tocotrienol;

5 FIGURE 5 is the chemical structure of 5-amino-d- α -tocopherol;

FIGURE 6 is the chemical structure of 4-[Fmoc-glutamide- α -OBzl- γ -(N-2,6-dimethylphenyl))amide];

FIGURE 7 is a schematic illustration of the synthesis of RRR-d- α -tocopheryl-6-amine;

10 FIGURE 8 is the chemical structure of 6-folyl tocopheryl-6-amine;

FIGURE 9 is the chemical structure of 6-methotrexyl tocopheryl-6-amine; and

FIGURE 10 is the chemical structure of a representative aminobenzopyran derivative of the invention, tocopheramine-tetraglutamate.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

15 In one aspect the present invention provides benzopyran derivatives, particularly aminobenzopyran derivative. In one embodiment, the benzopyran derivatives are amine derivatives. In another embodiment, the benzopyran derivatives are amide derivatives.

The chemical structures of representative benzopyran amine derivatives are illustrated in FIGURES 1, 2, 5, and 7-10. FIGURES 1A-D are chemical structures of
20 representative benzopyran amine derivatives of the invention. FIGURES 2A-D are chemical structures of representative benzopyran amide derivatives of the invention. FIGURE 5 is the chemical structure of 5-amino-d- α -tocopherol. FIGURE 7 provides the chemical structure of RRR-d- α -tocopheryl-6-amine. FIGURE 8 is the chemical structure of 6-folyl tocopheryl-6-amine. FIGURE 9 is the chemical structure of 6-methotrexyl
25 tocopheryl-6-amine. FIGURE 10 is the chemical structure of tocopheramine-tetraglutamate.

In these figures, the noted substituents are as described below.

R₁, R₂, R₃, and R₄ are substituents selected independently from hydrogen, hydrocarbyl, amino, hydroxyl, and carboxyl. In one embodiment, these substituents are
30 selected from hydrogen, C₁-C₄ hydrocarbyl, carboxyl, and hydroxyl. In another embodiment, the substituents are selected from hydrogen, methyl, and ethyl.

R₅ is a substituent that associates with water to form at least 2 hydrogen bonds, in one embodiment 3 hydrogen bonds, and in other embodiments from 2 to about 200

hydrogen bonds. R₅ can optionally form a salt in buffered water or saline, and may be selected from a carboxylate (such as sorbate, tartarate, succinate, citrate, gluconate, glucoheptonate, glycerate, itaconate, aconitate, galacturonate, galactarate, glutarate, creatine, fumarate and its polymers, ascorbate, lactate and its polymers, pangamate, pantothenate, or para-aminobenzoate), an amino acid, a purine (such as adenine and guanine), a nucleoside or nucleotide (such as adenosine, deoxyadenosine, guanidine, cytosine, thymidine, uridine, or polyadenosine), a polypeptide (such as vasopressin, polypep, ferrichromes, oxytocin, or rifampin), an alcohol (such as erythritol, adonitol, riboflavin, flavine-adenine-dinucleotide, glucovanillin, taurocholate, glycocholate, tauroursocolic acid, glyco-nor-ursodeoxycholic acid, thiol, glycerol, mannitol or cyanocobalamin), a sugar (such as glucosamine, n-acetylglucosamine, n-acetylneuraminate, lactose, ribose, arabinose, rhamnose, raffinose, maltose, lactobionose, heparin sulfate, trehalose, gluconate, galactose, sucrose or glucose), a polyhydric alcohol (for example $-(\text{OCH}_2\text{OH})_n\text{OH}$, $-(\text{OCH}_2\text{CHOH})_n\text{OH}$, $-(\text{OCH}_2\text{CHOHCHOH})_n\text{OH}$, $-(\text{OCH}_2\text{CH}_2\text{CHOH})_n\text{OH}$, $-(\text{OCH}_2\text{CHOH})_n\text{OH}$, $-(\text{OCH}_2\text{CH}_2\text{CHOH})_n\text{OH}$, or $-(\text{OCH}_2\text{CHOHCH}_2)_n\text{OH}$, where n is 1 to 100, and branched or block co-polymers of the same). In certain embodiments, R₅ includes residues of carnitine, sarcosine, taurine, methionine, glutathione, β-alanine, glycine, glutamate, glutamine, aspartate, asparagine, ornithine, arginine, γ-aminobutyrate (GABA), serotonin, adrenaline, histamine, melatonin, tryptamine, alanylglutamine, glycyglutamine, glycylysarcosine, valyl-lysine, aspartylalanine, glutamyltryptophane, lysyl-sarcosine, glycyproline, triglycine, polyglutamate (Glu)_n, polyglutamine (Gln)_n, polyglycine (Gly)_n, polyalanine (Ala)_n, polyproline (Pro)_n, poly-(GlyProAla)_n, polyserine (Ser)_n, and other biogenic amines as defined below, polyesters, copolymers of succinate, glycerol, and polyethylene glycol, polyhydroxyalkonates, polyhydroxypropionate, poly-(3-hydroxyvalerate), poly-(3-hydroxyhexanoate), poly-(4-hydroxyvalerate), poly-(5-hydroxyvalerate), generally R-3-hydroxyacid polymers and their derivatives, polyglycolides (PGA), polylactides (PLA), substituted polyhydroxybutyrates, folate, glycogen, chitosan, dextran, dextrin, gluconate, poly-N-substituted glycines, polyvinylpyrrolidinone, poloxamer, polyvinylalcohol, polyethylene glycol, l-amino-polyethyleneglycol, or composites (co-polymers) of the above.

In an alternate embodiment, R₅ provides a derivative that is not bonded or is weakly bonded by a C8 column packing, and most preferentially is not bonded or is

weakly bonded by a C18 column packing (for example BondElute™). Bonding can be assessed by measuring retention times on an HPLC set up with a reverse phase column and a gradient solvent system progressing from relatively nonpolar to more polar. Those compounds that are poorly retained (i.e., have low retention times) are the preferred compounds for R₅. Molecular weights for R₅ are typically between 20 Da to 5 kDa. In one embodiment, R₅ molecular weights are from about 80 Da to 5000 Da. In another embodiment, R₅ molecular weights are from about 180 Da to 2500 Da.

X is hydrogen or C₁ to C₆₀ hydrocarbyl or substituted hydrocarbyl, in which substituents may be one or more of, for example, hydroxyl, oxo, carboxy, halo, nitro, amino, sulfo, sulfhydryl, sulfate, or organophospho group. Preferably X is hydrogen, C₁ to C₄ hydrocarbyl or C₁ to C₆₀ polyhydric alcohol (for example -(OCH₂OH)_nOH, -(OCH₂CHOH)_nOH, -(OCH₂CHOHCHOH)_nOH, -(OCH₂CH₂CHOH)_nOH, -(OCH₂CHOH)_nOH, -(OCH₂CH₂CHOH)_nOH, or -(OCH₂CHOHCH₂)_nOH, where n is 1 to 100, and branched or block co-polymers of the same), and most preferentially hydrogen, (i.e., to form a secondary amine or amide), methyl, or ethyl (i.e., to form a tertiary amine).

T₁ is a C₁ to C₈₀ hydrocarbyl, hydroxyhydrocarbyl, carboxyhydrocarbyl, oxyhydrocarbyl, ketohydrocarbyl, oxohydrocarbyl, phosphohydroxy hydrocarbyl, saturated or unsaturated, branched or unbranched. In one embodiment, T₁ is an isoprenoid, terpene, diglyceride, or phospholipid. In another embodiment, T₁ is a phytyl (4,8,12-trimethyl-tridecyl) or trienyl (4,8,12-trimethyl-3,7,11-tridecatrienyl).

T₂ is hydrogen, or C₁ to C₈₀ hydrocarbyl, optionally substituted, saturated or unsaturated, branched or unbranched. In one embodiment, T₂ is a C₁ to C₁₈ hydrocarbyl group. In another embodiment, T₂ is methyl or ethyl. Alternatively, T₂ is a carboxyl group, wherein the carboxyl group is optionally esterified or amidated, respectively, with a C₁ to C₈₀ alcohol or amine, preferably a C₁ to C₁₈ alcohol or amine, and the alcohol or amine is optionally substituted, saturated or unsaturated, branched or unbranched, cyclical or acyclic.

The stereochemistry of T₁ and T₂ may be as d- or l-stereoisomers or as racemates, and the invention is not limited by the stereochemistry of any chiral centers.

The benzopyran derivatives can be cationic, anionic, zwitterionic, multipolar or nonionic. Ionically charged derivatives can be formed and used as salts, for example as

the sodium, hydrochloride, citrate, lactobionate, propionate, succinate, potassium, lithium or palmitate salt.

The invention further relates to compositions in which a pharmaceutical, nutraceutical, cosmeceutical, vitamin, foodstuff, antigen, catalyst, cell, nanoparticle, oligonucleotide, gene, extract, cosmetic or fiber is solubilized, protected or dispersed in a solution, particle, emulsion, microemulsion, nanoemulsion, liposome, niosome, molecular matrix or coating comprising one or more of the aminobenzopyran derivatives, optionally with other oils, co-solvents, surfactants and cosurfactants. In one embodiment, the composition is a biocompatible or therapeutic formulation comprising one or more aminobenzopyran derivatives for application to a human or to an animal by any of a variety of routes, including but not limited to oral, topical and parenteral administration. Such compositions include solutions, suspensions, emulsions, emulsion preconcentrates, liquigels, lotions, astringents, soaps, ointments, toothpastes, topicals, capsules, sustained release granules, powders, tablets, nosedrops, eyedrops, excipients, sunscreens, surgical dressings, intravenous infusions, depot or sustained release injections, and coatings for prosthetic devices.

Aminotocans, the aminobenzopyran derivatives of the invention, refer to select classes of derivatives of (5-, 6-, 7- or 8-amine)-1-benzopyran. The derivatives are formed by chemistry that is commercially attractive. The derivatives have unexpected utility as surfactants, as biocompatible surfactants, as pharmaceutical excipients, and as bioavailability enhancers. In contrast, typical commercial surfactants such as sodium oleate (a principal component of soap), betaine, or SDS (sodium dodecyl sulfate) dissolve biological membranes and are corrosive to cells, so the utility of surfactants that, almost paradoxically, serve to stabilize biocellular membranes is anticipated to be great.

The benzopyran derivatives displayed surfactant properties of foaming and emulsion formation even before the protective groups on the "hydrophilic head" had been removed.

The biocompatibility of tocol oils in contact with cell membranes and organelles is truly remarkable, and it is well known that tocols characteristically stabilize biological membranes in the presence of other surfactants. The biocompatible benzopyran derivatives are surfactants that may be anionic, cationic, zwitterionic, multipolar, or non-ionic.

To assist in understanding the invention, the following definitions are provided.

Vitamin E: Vitamin E as used herein is the common name for RRR- α -tocopherol (d- α -tocopherol, *sensu stricto* 2,5,7,8-tetramethyl-2-(4',8',12'-trimethyltridecyl-6-benzopyranol), the vitamin named by Dr. George Calhoun and Dr. H.M. Evans in 1936. The suffix "-ol" denotes the presence of the 6-hydroxyl on the benzopyran ring. Vitamin E is a member of the family "tocopherols." Vitamin E has a bioequivalence of 1.00 α TE units in biological assays for Vitamin E activity.

Tocopherol(s): Tocopherols are a family of natural and synthetic compounds containing three key structural elements, a benzopyran ring, phenolic alcohol, and phytol tail. Vitamin E is an important representative of the tocopherol family, and its molecular structure is shown in FIGURE 3. Not all tocopherols have three methyl groups on the chroman head. The simplest family member contains no methyl groups on the chroman ring, 6-hydroxy-2-methyl-2-phytylchroman), and is sometimes simply referred to as "tocol", although the term "tocol" is used herein to represent all tocopherols and tocotrienols. There are four tocopherol family members that are commonly encountered in food and natural products, and eight possible isomers in total (not including stereoisomers). The names of the family members are shown in the table below.

Position of methyl groups on chroman head	Tocopherol family common name
5,7,8	α -tocopherol
5,8	β -tocopherol
7,8	γ -tocopherol
5,7	ξ_2 -tocopherol
8	δ -tocopherol
5	5-methyltol, " ϵ_1 -tocopherol"
7	η -tocopherol
0	"tocol"

It is important to recognize that all tocopherols share the phenolic alcohol as a functional group at the 6-position on the chroman head, regardless of the position of the methyl groups. In addition, the R/S stereoisomers described for the phytyl tail of α -tocopherol (3 chiral centers, 8 isomers in all) are also present in each of the other tocopherol families, e.g., beta, delta and gamma. Thus the total number of natural molecules named in the table, including stereoisomers, is $8 \times 8 = 64$.

Tocotrienol(s): Tocotrienols have structures related to the tocopherols, but possess a 3', 7', 11'-triene "tail" at the 2-position on the benzopyran ring. For illustration, the structure of d- α -tocotrienol is shown in FIGURE 4. Again, as is the case for the tocopherols, not all tocotrienol family members have three methyl groups on the chroman head. There are four family members that are commonly encountered in food, and eight possible members in total, more if the desmethyl forms are considered.

Tocotrienol nomenclature is not fully consistent at the level of common names. However, all tocotrienols share the phenolic alcohol at the 6-position on the chroman head. Adding another layer of complexity, the double bonds at the 3, 7, and 11 positions of the tail may be "cis" or "trans", but typically are all-trans in the natural products.

Interestingly, the δ -tocopherol and δ -tocotrienols are some of the best antioxidants of the group.

Tocol-soluble: Refers to the property of certain molecules characterized as being soluble directly, or with the aid of a co-solvent, in a tocol. As an operative definition, the most useful way to determine tocol solubility is to dissolve the compound of interest in a tocol or to use a co-solvent such as ethanol.

U.S. Patent Application No. 09/671,753 filed September 27, 2000, and PCT application PCT/US00/26467, (both of which are hereby incorporated herein by reference) disclose formation of tocol-soluble ion pairs between charged tocol derivatives and oppositely charged therapeutic compounds. By "ion pair" is meant a neutral pair formed between two oppositely charged compounds. Compounds of the present invention are among the tocol derivatives that are capable of forming tocol-soluble ion pairs with such oppositely charged therapeutics, thereby rendering them soluble in tocols or enhancing existing tocol solubility. The resulting compositions can be incorporated into various types of pharmaceutical compositions, including multiphasic compositions or their precursors, such as emulsions, liquid crystalline gels, self-emulsifying drug delivery

systems, or liposomal or niosomal dispersions, for oral or other (including parenteral) administration.

Tocan or Tocans: "Tocan" or "tocans" are used herein in a broad sense to indicate the various members of the families of tocopherols and tocotrienols, their rarer natural and synthetic analogs, and in addition all benzopyran derivatives substituted at the 2-position by T_1 and T_2 , where T_1 is a C_1 to C_{80} hydrocarbyl, hydroxyhydrocarbyl, oxyhydrocarbyl, carboxyhydrocarbyl or phosphohydroxy hydrocarbyl, saturated or unsaturated, branched or unbranched, an isoprenoid, terpene, diglyceride, phospholipid, a phytyl (4,8,12-trimethyl-tridecyl), or trienyl (4,8,12-trimethyl-3,7,11-tridecatrienyl), and 10 T_2 is a hydrogen, halo, hydrocarbyl, carbonyl, methyl, ethyl, or carboxyl, as the 1-stereoisomer.

Tocans also include tocols esterified at the 6-hydroxyl on the benzopyran ring. When administered *in vivo*, these derivatives are readily de-esterified at low pH or by esterases in the thoracic duct and in the blood, releasing the free tocol. Common tocol 15 esters known in the art include the acetate, succinate, maleate, phosphate, linoleate, nicotinate, ascorbate, retinoate, quinone, and a pegylated diester derivative known as TPGS (tocopherol polyethylene glycol succinate).

A special case is the benzopyran derivative known as 6-hydroxy, 2,5,7,8-tetramethylchroman-2-carboxylic acid (commercially available as Trolox[®]), which has a 20 reactive carboxyl functional group at the two position and is relatively hydrophobic despite lack of a lipid sidechain at T_2 . Tocol desmethyl analogs have been isolated or synthesized. Other synthetic tocols include Raloxifast.

d- α -Tocopherol-5-amine: has the chemical structure shown in FIGURE 5.

Linker: In chemical synthesis, conjugations of two molecules A and B may take 25 place using a linker "C" to modify the reactivity of a functional group so that the joining takes the form A+C+B in the final structure. Note the position of C between A and B. Linkers may be homo- or heterobifunctional, or multifunctional as in the synthesis of dendrimeric molecules.

Spacer: A spacer is a special class of linkers in which the separation of A and B is 30 increased by the length of the spacer C.

Cap: An end group on a side chain, particularly on a polymer.

Surfactant: Surfactants are bipolar molecules characterized by one simple property: they are driven to occupy interfaces between two phases, typically either

liquid/gas phase interfaces, liquid/solid interfaces, or liquid/liquid phase interfaces (for immiscible liquids), so that the free energy of the boundary surface between phases is reduced. Obviously, the "surfactanticity" of any molecule is not independent of the properties of the immiscible phases under study. With respect, for example, to water and oil, a surfactant will contain both a water-loving "head" and an oil-loving "tail". Other surfactants, however, have fluorophilic "tails." The basic principle is that the molecule is amphipolar with respect to the two phases with which it associates. Surfactants may be classed into five sub-groups: anionic, cationic, zwitterionic, multipolar and non-ionic on the basis of chemical structure and the presence or absence of electrostatically charged substituents. Surfactants are also sometimes termed "emulsifiers." Catanionic, gemini, and bolaform surfactants are special cases.

Co-surfactant: A second surfactant that aids in reduction of the surface free energy between two phases, most typically that aids in fine emulsification of an oil and water or dirt and water system.

Hydrophile-Lipophile Balance (HLB): Is an empirical formula used to index the relative detergency of surfactants. Its value varies from 1 to about 45 and in the case of non-ionic surfactants from about 1-20. In general, for lipophilic surfactants the HLB is less than 10 and for hydrophilic ones the HLB is greater than 10.

Critical Micellar Concentration (CMC): Is an experimentally determined concentration of amphiphile, surfactant or detergent molecules in solution distinguished by the appearance of organized "micelles" (defined below).

Oil: Any of a class of hydrocarbon derivatives that are hydrophobic and immiscible or poorly miscible with water. They may be synthetic or derived from plants, animals or microorganisms. Such oils include "grease," waxes, "dirt," triglycerides, diglycerides, derivatives of mono- and diglycerides, essential oils, Vitamin oils, nutrient oils, squalene, squalane, waxes, terpenes, ethers and crown ethers, and may be either synthetic or natural. In general, the melting points of oils are less than 100°C and most are in fact liquid at body temperature.

Common oils include extracted and distilled oils from nuts and seeds, for example safflower, perrila, millet, niger, Ucuúba, sesame, cimhopogon, mustard, canola, corn, caraway, soybean, sunflower, garlic, peanut, pumpkin seed, olive, almond, macadamia, palm, walnut, pistachio, coconut, evening primrose seed, black currant seed, rosemary, borage seed or flax seed oils, and from fish and phytoplankton, for example shark, cod,

mackerel, sardine, salmon, scrod, or halibut oils, or from oleagenous microorganisms directly. Also included are tocots (comprising the whole family of tocopherols and tocotrienols, including the acetate esters), certain terpenoids comprising Vitamin A (also called retinol), retinoids, menaquinones such as Coenzyme Q, carotenoids such as carotenes, lycopene, and the related xanthophylls such as lutein, lutein esters, astaxanthin, canthaxanthin and zeaxanthin, Vitamin D, Vitamin K, vitamers of these Vitamin oils and their precursors, and glycerides high in PUFAs such as triglycerides containing esterified docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Also contemplated as oils are essential oils. These are often complex mixtures useful in enhancing bioavailability, and include extracts of (as in U.S. Patent No. 5,716,928) allspice berry, fennel, amber essence, anise seed, arnica, balsam of Peru, basil, bay leaf, parsley, peanut, benzoin gum, bergamot, rosewood, rosemary, rosehip, cajeput, marigold, turmeric, camphor, caraway, cardamom, carrot, cedarwood, celery, chamomile, cinnamon, citronella, palm kernels, avocado, macadamia, sage, clove, coriander, cumin, cypress, eucalyptus, aloe, fennel, fir, frankincense, garlic, geranium, rose, ginger, lime, grapefruit, orange, hyssop, jasmine, jojoba, juniper, lavender, lemon, lemongrass, marjoram, mugwort, watercress, mullen, myrrh, bigarde neroli, nutmeg, bitter orange, oregano, patchouly, pennyroyal, primrose, retinols, papaya, red pepper, black pepper, baccharis (Vassoura Oil), peppermint, poppyseed, petitegrain, pine, spruce, poke root, rosemary, sandalwood, saffron, spearmint, spikenard, hemlock, tangerine, tea tree, thyme, vanilla, banana, coconut, vetivert, wintergreen, witch hazel, ylang ylang extract, or synthetic analogs.

Colloidal System: As used herein, this term refers to a system containing two or more immiscible phases, at least one of which takes the form of a particle or droplet and is termed a "dispersed" phase, and one phase is a liquid or a solution and is termed a "continuous" phase. As used herein, "colloidal systems" are limited to those wherein the particles are larger than simple molecular or micellar solutes but are small enough that they remain suspended in a fluid medium without settling to the bottom. The vapor pressure of a liquid or solution containing a colloid is typically not influenced by the colloidal particles or droplets in suspension. Nor are other colligative properties affected, for example osmolarity. A review of the prior art of colloidal systems is provided in Zograf, G. et al. 1990 "Disperse systems" in (Gennaro, A.R. and T. Medwick, eds.) *Remington's Pharmaceutical Sciences*, Philadelphia, PA. Emulsions, nanoemulsions,

mini-emulsions, and liposomes are examples of colloidal suspensions. Oil-in-water (o/w), water-in-oil (w/o), solid-in-oil, solid-in-water, and oil-in-solid colloidal systems are known in the art. Colloidal systems are most preferably stabilized with surfactants. Aspects of the invention also include precursors of colloidal systems, for example, 5 SEDDS (self-emulsifying drug delivery systems), wherein the oily phase containing a therapeutic agent is administered as a preconcentrate, typically with surfactant(s), sometimes with solvents, but absent water.

Multiphase System: As used herein, this term refers to a system where one or more phases is (are) dispersed throughout another phase, which is usually referred to as 10 the continuous phase, or a precursor thereof. Complex emulsions, microemulsions and other multiphase nanoparticulates, including liposomes, niosomes and crystalline suspensions in oil-in-water emulsions, are examples of multiphase systems. These systems may be lyophilic or lyophobic. Biphasic systems are a subcategory of multiphase systems.

15 Emulsion: A colloidal dispersion of two immiscible or poorly miscible liquid phases, such as oil and water, in the form of droplets. The internal phase is also termed the dispersed phase and the external phase is termed the continuous phase. The mean diameter of the dispersed phase, in general, is between about 0.2 and about 50.0 microns (μm), as is commonly measured by particle sizing methods, and the particles range 20 broadly in size. Emulsions in which the dispersed phase and continuous phase have different refractive indexes are typically optically opaque. Emulsions in which the refractive indexes of the two phases are similar may be clear or translucent, and hence optical appearance is not a defining characteristic. Emulsions possess a finite or limited stability over time, and can be stabilized for days or weeks, sometimes for months, by the 25 incorporation of surfactants and by viscosity modifiers.

Microemulsion: A thermodynamically stable, isotropically clear mixture of two immiscible liquids, stabilized by a relatively high concentration of surfactant molecules. Microemulsions have an apparent mean droplet diameter of less than about 200 nm, in general from about 10 to about 100 nm, and are typically self-assembling, or may be 30 assembled using heat and/or solvents. Typically, microemulsions are more easily established with a co-surfactant. Microemulsions exist only within defined ratios of water, amphiphile and oil, as may be determined with a phase diagram for the system at a given temperature. Therefore, o/w microemulsions are inherently unstable when diluted

with water. A class of microemulsions known as "swollen micelles" constitutes a system of special interest for drug delivery. Microemulsions of non-volatile oils decrease the vapor pressure of the continuous phase, and conversely, microemulsions of volatile oils may increase the vapor pressure of the continuous phase, a change that may involve
5 substantial departures from ideal solute behavior, where ideality is taken as an approximately linear relationship between solute concentration and vapor pressure.

Tocan Microemulsion: A thermodynamically stable, isotropically clear mixture of two immiscible liquids, one of which contains a tocan oil or tocan surfactant, stabilized by a relatively high concentration of surfactant molecules. Tocan microemulsions have
10 an apparent mean droplet diameter of less than about 200 nm, in general from about 10 to about 120 nm. Once manufactured, however, they are filter sterilizable and are highly stable in a defined temperature range. Typically, tocan microemulsions are more easily established with a co-surfactant. A class of tocan microemulsions known as "swollen micelles" constitutes a system of special interest for drug delivery.

Nanoemulsion: Nanoemulsions, sometimes termed miniemulsions, are colloidal systems distinct from microemulsions and emulsions. As used herein, this term refers to those systems having a mean particle size that is less than 200 nm (as Gaussian volumetric mean), preferably less than 120 nm, and most preferably from about 10 to 100
15 nm, and not displaying apparent growth in particle size as measured by a lack of increase in size of greater than 15% in Gaussian volumetric mean by photon correlation spectroscopy when incubated at 25°C under controlled conditions for at least 30 days, preferably for 6 months, and most preferentially for up to 2 years. Some of these vehicles are isotropically clear and display high levels of drug loading (a relative property that must be determined independently for each drug). Some are translucent or hazy. Some
20 nanoemulsions are self-assembling, but others may require heat, solvent, and/or increased shear to assemble due to the high viscosity of certain nutrient oils. Operationally, nanoemulsions, however formed, share the common property of being terminally filter sterilizable, typically by passage through a compatible filter membrane of a pore size not to exceed 0.2 microns. And unlike o/w microemulsions, o/w nanoemulsions are robust
25 when diluted with aqueous IV solutions, an important property when used for drug delivery. In general, nanoemulsions are differentiated from microemulsions by their behavior upon dilution and from the broader category of emulsions by their size and
30

stability. The relative insensitivity of the vapor pressure of the continuous phase to the presence of a nanoemulsion in suspension is a distinctive characteristic.

Tocan Nanoemulsions: Tocan nanoemulsion drug delivery vehicles contain a tocan surfactant or oil, optionally a cosurfactant, and an oil or oils and have a mean
5 particle size that is less than 120 nm (as Gaussian volumetric mean), preferentially less than 100 nm, and do not display apparent growth in particle size (as measured by a lack of increase in size of greater than 15% in mean diameter by photon correlation spectroscopy) when incubated at 25°C under controlled conditions for at least 30 days, preferentially for 6 months, and most preferentially for up to 2 years. Many of these
10 vehicles are optically translucent or clear, but display high levels of drug loading (a relative property that must be determined independently for each drug). Some tocan nanoemulsions are self-assembling, but others may require heat, solvent, and/or increased shear to formulate due to the high viscosity of certain tocan oils. Operationally, tocan nanoemulsions share the common property of being terminally filter sterilizable, typically
15 by passage through a compatible filter membrane of pore size 0.2 microns. Tocan oil nanoemulsions are further characterized by modest or negligible effect on the vapor pressure of the continuous phase.

Liposome: A lipid bilayer vesicle formed spontaneously upon dispersion of certain lipids, most commonly phospholipids, in water. "Liposome" is also defined as a
20 vesicular structure consisting of hydrated bilayers.

Tocan Liposome: A bilayer vesicle formed spontaneously upon dispersion of certain highly polar tocans in water. "Liposome" is also defined as a vesicular structure consisting of hydrated bilayers.

Niosome: By analogy to a liposome, a niosome is a nonionic surfactant vesicle.
25 Classes of commonly used non-ionic surfactants include polyglycerol alkylethers, glucosyl dialkylethers, crown ethers and polyoxyethylene alkyl ethers and esters.

Tocan Niosome: By analogy to a liposome, a tocan niosome is a nonionic tocan surfactant vesicle.

Micelle: These are organized dynamic molecular aggregates of one or more
30 surfactants that exist only at a concentration above the critical micellar concentration (CMC) in water or buffer. The CMC is an individual characteristic of each surfactant. These molecular aggregates typically have a nominal diameter of 2 to 6 nm, and perhaps 15 or 20 nm in some systems. Micellar solutions of surfactants cause departures from

non-ideality of the vapor pressure of the continuous phase and have other properties not characteristic of colloids.

Self-Emulsifying Drug Delivery Systems (SEDDS): With reference to a phase diagram, certain mixtures of oil(s) and non-ionic surfactant(s) will form clear and isotropic solutions that then spontaneously emulsify when mixed with water. These mixtures, when comprising drug as well as oil and surfactant, are known as self-emulsifying drug delivery systems (SEDDS). Optionally, they may also contain solvents and other excipients. SEDDS and the related SMEDDS (self-microemulsifying drug delivery systems) have successfully been used to improve lipophilic drug dissolution and oral absorption.

Tocan Self-Emulsifying Drug Delivery Systems (TSEDDS): With reference to a phase diagram, certain mixtures of oil(s) and tocan surfactant(s) will form clear and isotropic solutions that then spontaneously emulsify when mixed with water. These mixtures, when comprising drug as well as oil and surfactant, are known as self-emulsifying drug delivery systems (SEDDS). Optionally, they may also contain solvents and other excipients. TSEDDS and the related TSMEDDS (tocan self-microemulsifying drug delivery systems) are useful to improve lipophilic drug dissolution and oral absorption.

Biocompatible: Capable of performing functions within or upon a living organism in a manner that does not terminate or excessively disable the life of the organism, i.e., without *undue* toxicity or harmful physiological or pharmacological effects.

Prodrug: A prodrug is a chemical derivative of a therapeutic agent which, following administration, is cleaved or metabolized to release the therapeutic agent *in situ*.

Hydrocarbyl: By "hydrocarbyl" is meant moieties containing carbon and hydrogen atoms only, with the indicated number of carbon atoms. Hydrocarbyl groups may be straight-chain or branched-chain, aliphatic or aromatic, alkanes or alkenes. Unsaturated groups such as 1- and 2-butene and propargyl, including multiple unsaturated groups such as butadienyl and phenyl or polyphenyl, are included in this term.

In one aspect, the present invention provides emulsion or liposome particles coated with a "stealth coat" so as to evade the phagocytic cells of the reticuloendothelial cell system (RES), comprising the liver, lungs, spleen and bone marrow. In one

embodiment, particles that target particular cells where the therapeutic agent is needed, for example cancer cells.

A variety of polyoxyethylated derivatives have been used to form the "stealth coat" around the particles or liposomes. Examples of these agents include
5 POLOXAMERS (also termed Pluronic[®]), which are block co-polymers of polyoxyethylene and polyoxypropylene, and pegylated surfactants such as Tween 80 and pegylated phospholipids. Particles coated with these molecules have been somewhat successful in evading the RES, but because of inherent toxicity or expense, have been less than satisfactory in clinical use. Furthermore, their corrosive detergency has rendered
10 these compounds of little use in cosmetic applications.

Tocopherol, because of its gentle ability to stabilize biological membranes, is an excellent starting material to form a "stealth coat" surfactant for drugs and cosmetics. However, the chemical reactivity of the phenolic hydroxyl of tocopherol is limited and the ability to synthesize and screen tocol conjugates is substantially hampered by the
15 strong reactants required to form a bond with the hydroxyl. Frequently, unacceptable side reactions occur or no reaction at all.

A related compound, d,l-6-tocopheramine, which has been described in the literature, has been overlooked as a platform for the formation of prodrug and surfactant conjugates. This compound possesses excellent reactivity and versatility for synthesis of
20 complex derivatives. The membrane insertion and stabilizing properties of this compound are expected to be retained and also expected to be biodegradable, albeit more slowly than esters or phosphate diesters. Furthermore, aminotocans that retain the 6-hydroxyl were expected to be effective antioxidants.

The present invention provides benzopyran derivatives, including aminotocans
25 (and other tocans) as a platform for covalently coupling biomolecules to benzopyrans. In one embodiment, "stealth coat" surfactants comprising a hydrophilic "head" and a phytol tail are provided. The very "greasy" phytol or phytotrienyl tail can be used to position correspondingly large hydrophilic heads at a lipid/water interface. Whereas stearylamine has a molecular weight of 270 daltons, the analogous aminotocan has a molecular weight
30 of about 450 daltons, a 65% increase. Betaine, a good example of a corrosive and toxic detergent, has a CMC of about 0.0006 M, the CMC of TPGS is 0.0001 M, making TPGS a better detergent by a factor of six at low concentrations while gentle on cells. The large hydrophilic heads can also be used to prevent the interaction of the lipid droplets with

phagocytic cells by "steric hindrance" and by binding of a layer of water to the particle through hydrogen bonds. In one embodiment, the benzopyran derivative is an aminotocan polyglutamate. Surfactants of this type are hydrophilic with high HLB values and are effective by steric hindrances to coalescence and by electrostatic repulsion in stabilizing emulsions during storage. These derivatives have excellent detergency but are gentle on biological membranes.

Polyglutamate is an effective "stealth coat." *Bacillus anthracis*, a highly virulent bacterium which multiplies unchecked in the blood stream of mammals, including man, uses a capsule of polyglutamate (gamma-linked) to avoid triggering an immune response and to avoid phagocytosis. Thus, an artificial particle encapsulated in a polyglutamate coat could also evade the immune system, leading to prolonged circulation in the bloodstream, a highly desirable outcome. In the invention, polyglutamate is covalently coupled to an appropriately substituted benzopyrans via mixed anhydride chemistry.

The primary advantage of selected aminotocans as a starting material for formation of tocan derivatives is the highly chemically reactive character of the amine, particularly for nucleophilic substitutions. Conceptually, this is a major advance over prior art, wherein substituent molecules are coupled to Vitamin E via a linker. This is because of the limited reactivity and strong reaction conditions required to render reactive the phenolic hydroxyl of tocopherols, chemistry which is known to be difficult or of limited versatility because of the relatively poor nucleophilicity of the phenolic hydroxyl and its rapid oxidation to yield undesirable chromogenic quinones (Skinner, W.A. and R.M. Parkhurst, 1970, "Reaction products of tocopherols" *Lipids* 6:240-44).

Generally, the addition of the biomolecule or targeting agent (see, e.g., R₅ in FIGURES 1 and 2) is the last step of any synthesis. The first steps involve formation of the tocan and functional group (amine). This is preferentially done directly from a preferred starting material, often a natural product, so that the stereochemistry of the product can be preserved. For example, Vitamin E or Trolox is deoxygenated at the 6-position and then nitrated and reduced so as to form an amine at the 6-position to form 6-tocopherol amine or 2,5,7,8-tetramethylchroman,-2-carboxylic acid, -6 amine, respectively. Alternatively, δ -tocotrienol may be aminated by direct metallation or through a bromine intermediate to yield mixtures of 5-, 7-, and 5,7-d- δ -tocotriene amine. Our experience has shown that the 5-position is most reactive, comprising as much as 80% of the product.

Other processes known in the art for producing the d,1- α -tocopherol 6-amine include condensation of phytol to 2,3,5-trimethyl-4-aminophenol and its formyl derivative (Smith, et al., *J. Am. Chem. Soc* 64:1082-84; 1942) and condensation of 2,3,5-triacyl-4-aminoacylphenol with isophytol or dihydrolinalool, in both cases forming the d,1
5 racemic mixture (Schlegel et al., U.S. Patent No. 3,458,637 and Schwieter, Swiss patent 463534).

Following introduction of the amine, the R₅ group is then added to form the desired aminobenzopyran derivative. The chemistry of aminobenzopyran derivatives may preferentially involve "amide" (also called "peptide") bonds of the form NH-CO-.
10 The amide bonds are preferentially formed by mixed anhydride chemistry, or may be formed with carbodiimides or phosgene as a condensing agent. The preferred amide bonds are biodegradable, but are more slowly degraded than ester, sulfhydryl, or diphosphoester bonds, and for that reason have surprising advantages as surfactants.

Regarding the chemistry of the aminotocan conjugates, the chemical bond is
15 preferably a "zero linker" amide bond. The active functional group is the 5, 6, 7, or 8 amine on the benzopyran ring. However, by attaching bifunctional or multifunctional linkers such as glycine, glutamate, aspartic acid, cysteine, lysine, arginine, or even succinate to the amine, additional functional reactivity is obtained. Both homomultifunctional and heteromultifunctional linkers are provided.

20 During derivatization, delicate substituents must be protected from chemical degradation with "protective groups," which are subsequently cleaved off under mild conditions. For this reason, strong reaction conditions are impractical, a factor that rules out many common synthetic pathways. Mild or highly specific chemical synthetic routes that are practical often lack the yields required to be commercially desirable. Because of
25 these limitations, the use of tocopherol as starting material for preparation of drug conjugates has been limited and highly specialized. No facile approach to tocopherol-drug conjugation has emerged despite 50 years of research. Given these requirements, the mixed anhydride chemistry of amide bonds for conjugation has clear advantages in terms of specificity, cost and yield.

30 Tocans as surfactants, for example as soaps, detergents and cosmetics, will have good environmental compatibility, be biodegradable, be gentle on the skin or mucosa, be active in hard water, will absorb UV radiation, will adhere to skin, hair or fiber, and will

be readily manufacturable. These are requirements that are addressed by the present invention.

The solvent and surfactant properties of aminotocans, and their derivatives, for cosmetics and medicament delivery vehicles has not previously been evaluated. Tocans
5 can serve as biological detergents or as depot storage forms for the sustained-release delivery of drugs. Other multiphase, systems include liquid crystalline structures such as liposomes, which are also suitable for drug delivery. In contrast, the relatively nonpolar tocopherols themselves have proved rather poor in forming liposomes, and only 20 mol% tocopherol could be incorporated into liposomes made from phospholipids (Fukuzawa K.
10 et al. 1992. Location and dynamics of α -tocopherol in model phospholipid membranes with different charges. *Chem Phys Lipids* 63:69-75), with little surface association of the phenolic hydroxyl. Aminotocans are particularly useful in forming cationic surfactants and cationic liposomes. Similarly, ionic nanoparticles or nanocrystals are provided.

In another aspect, the invention provides compositions in which a pharmaceutical,
15 nutraceutical, cosmeceutical, vitamin, foodstuff, antigen, catalyst, cell, nanoparticle, oligonucleotide, gene, extract, cosmetic or fiber is solubilized, protected or dispersed in a solution, particle, emulsion, microemulsion, nanoemulsion, liposome, niosome, molecular matrix or coating that include the benzopyran derivatives of the invention, optionally with other oils, co-solvents, surfactants and co-surfactants. Also described are specific
20 examples of "zero linker" and linker-based aminotocans and aminobenzopyrans, and their derivatives, and the uses of aminotocans as excipients, surfactants, as prodrugs, and as novel therapeutics, in creams, lotions, soaps, cosmetics, sunscreens, eyedrops, foodstuffs, toothpaste, detergents, emulsions, microemulsions, liposomes, capsules, nanosuspensions, nutraceuticals and injectables. The compositions may be administered
25 orally, topically, or by other nonparenteral routes.

Regarding compositions containing the benzopyran derivatives of the present invention, many therapeutically useful drugs or other compounds are insoluble in water or poorly soluble, and must be administered in the form of an emulsion, microemulsion, or pre-emulsion concentrate, or as a micellar solution or liposome. By modifying the
30 surface property of the emulsion droplet or liposome, the present invention provides a targeted therapeutic agent that can be direct to the desired cell. These tocan derivatives can be used to modify the surface properties of emulsion droplets or liposomes so as to produce "stealth" or targeted medicament delivery formulations.

In another embodiment, the composition of the invention is a liposome that includes a tocan or derivative selected for its chemical stability and its ability to form bilayers alone and in mixtures with phospholipids and cholesterol or phytosterols. The tocans of the present invention form liposomes with novel surface properties.

5 In a further embodiment, targeted benzopyran derivatives are provided. The hydrophilic substituent of the tocan surfactant is selected from a list of compounds, such as folate, that are preferentially bound, adhere to, or are taken up by particular types of cells. In this way, the contents of the drug delivery vehicle can be targeted to these cells. See, e.g., Reddy, J.A. and P.S. Low, 1998, "Folate-mediated targeting of therapeutic and
10 imaging agents to cancers" *Crit. Rev. Therapeutic Drug Carrier Systems* 15(6):587-627.

The benzopyran-containing compositions of the invention can be in the form of an emulsion, microemulsion, micellar solution, liquid crystalline system, self-emulsifying drug delivery system, or a liposomal formulation for parenteral administration. As used herein the term, "parenteral administration" includes intravenous, pulmonary, intraocular,
15 intrathecal, transmucosal, intratracheal, transdermal, subcutaneous, intraperitoneal or intramuscular administration. Oil-in-water, water-in-oil, and bicontinuous emulsions (Shinoda, K. et al., 1984, "Principle of attaining very large solubilization" *J. Phys. Chem.* 88:5126), nanoemulsions, microemulsions, as well as liposomes, soaps, and detergents are suitable forms of the invention.

20 The following examples are provide for the purpose of illustrating, not limiting, the invention.

EXAMPLES

Example 1

The Synthesis of 4-[Fmoc-glutamide- α -OBzl- γ -(N-2,6-dimethylphenyl))amide]

25 In this example, the synthesis of 4-[Fmoc-glutamide- α -OBzl- γ -(N-2,6-dimethylphenyl))amide] is described.

As a demonstration, the synthetic kernel of an aminotocan derivative, 4-glutamyl-2,6-dimethylaniline, was synthesized as described here. First, the "mixed anhydride" of the protected amino acid was prepared by reacting 1.2 eq. of IBCF and 1.7 eq of NMM with 1.0 eq of Fmoc-glutamic- α -OBzl- γ -OH in tetrahydrofuran (THF) at -5°C for about
30 60 minutes. The (NMM:HC1) was filtered off and the resulting Fmoc-glutamic- α -OBzl- γ -mixed anhydride was added dropwise to a solution of 1.0 eq. of 2,6-dimethylaniline and 1.3 eq. TEA dissolved in tetrahydrofuran at -5°C for 30 minutes. The mixture was

allowed to slowly warm to room temperature and stirred for an additional 20 hours. Additional 2,6-dimethylaniline (0.9 eq.) was added and stirred for reaction mixture for 1 hour. Finally, the reaction was heated to 57-58 °C and stirred for about 2 hours. The THF was then removed under vacuum to yield the crude product. Dichloromethane (DCM) was added to extract the product, and washed with two times with 0.1N HCl, two times with saturated NaHCO₃, and one time with saturated brine. The resulting organic layer was dried over anhydrous MgSO₄, filtered and dried down under vacuum to yield an off-white solid, with a yield of 50% by weight starting material. The aniline derivative was recrystallized from DCM with a 97% purity. FT-IR: amide, ester, carbamate, amide (N-H, C=O, C=O, C=O) 3288, 1739, 1696 and 1653 cm⁻¹, respectively. The product is illustrated in FIGURE 6.

Example 2

The Synthesis of a Representative Benzopyran Derivative:

RRR-d- α -Tocopheryl-6-amine

In this example, the synthesis of a representative benzopyran derivative of the invention, a 6-aminotocopherol, is described.

The reported syntheses of α -tocopheramine (Smith, L.I.; Renfrow, W.B.; Opie, J. 1942. *J. Am. Chem. Soc.* 64:1082-1084; Ismail, F.M.D.; Hilton, M.; Stefinovi'c, M. 1992. *Tetrahedron Lett.* 33:3795-3796) are modifications of the original route used to assemble α -tocopherol (Vitamin E). Specifically, the appropriate phenolic compound is condensed with a synthetic or natural phytol. These routes are problematic because the chemistry would eventually prove difficult to scale (e.g., diazonium chemistry), the overall yield is low (primarily due to the final purification), and the stereochemistry is not specific for the natural RRR-d- α -tocopherol (or its tocopheramine stereoanalog). In short, the process is not commercializable.

Conversion of α -tocopherol to α -tocopheramine is depicted in FIGURE 7. The advantage of this route is significant: all the necessary carbons and connectivity are present in the starting material and the biologically unique stereochemistry is preserved.

Deoxygenation of phenols begins by activating the phenolic oxygen as some derivative, such as tosylate, isourea, dimethylthiocarbonate, or triflate, followed by reduction (Sebok, P.; Timar, T.; Eszenyi, T. 1994. *J. Org. Chem.* 59:6318-6321; Wang, F.; Chiba, K.; Tada, M. 1992. *J. Chem. Soc. Perkin Trans. 1* 1992:1897-1900; Saa, J. M.; Dopico, M.; Martorell, G.; Garcia-Raso, A. 1990. *J. Org. Chem.* 55:991-995. The

most practical methods is activation as a triflate and then either catalytic hydrogenation (Raney nickel) or chemical reduction (NaBH_4 , NiCl_2). Tosylate activation is less expensive than triflate but triflate was more successful with the hindered phenol.

Reduction of Phenol Derivatives.

5

Derivative	Conditions	Reference	Observations
-SO ₂ To1	H ₂ , Raney Ni NaOH, 5 ahn OR NaBH ₄ /NiCl ₂	3,4	economical and scalable
-CNHNEt ₂	H ₂ /Pd on carbon EtOH, RT, 5 atm	3	three-step sequence
-SCNMe ₂	Raney Ni EtOH, RT	3	four-step sequence
-SO ₂ CF ₃	H ₂ /Pd on carbon H ₂ /(Ph ₃ P) ₂ PdCl ₂ / Bu ₃ N/DMF	5	compatible with highly substituted phenols

The strategy was to use the most promising deoxygenation reactions and subsequently carry on to the final steps of nitration and reduction to α -tocopheramine. Following deoxygenation, nitration was effected under very mild conditions with no selectivity issues. For reduction to an amine, there were a variety of mild conditions successful in converting the nitroaromatic to an aniline. The product was purified and determined to be RRR-d- α -6-tocopheramine.

Note that the amine could optionally be placed at the 5 or 7 position by starting with a benzopyran compound open on the aromatic ring at that position, for example δ -tocotrienol or δ -tocopherol, and performing the nitration and reduction to an amine without need for deoxygenation.

Amines that are starting materials for the novel aminobenzopyran compounds of this invention, other than d,l- α -6-tocopheramine, are novel compounds. Similarly, starting materials having both an amino- and a hydroxyl- substituent on the ring, are also novel compounds. Other novel compounds of this invention include intermediates such

as the nitro-substituted intermediates as depicted in the above process, and amido compounds used in preparing those novel benzopyran derivatives that are N-substituted amides. These novel compounds, as well as the process described above, constitute aspects of the overall invention.

5 Prior art processes were capable of producing only the mixed racemate of 6-tocopheramine (i.e., the d,1- α -tocopheramine as the mixed stereoisomer) and techniques for resolving or separating the individual tocopheramine isomers have not been disclosed. The above process, however, is capable of maintaining the stereochemistry of the starting tocol, for example the RRR-d- α -tocopherol or RRR-d- α -
10 tocotrienol). Consequently, the optical isomers produced using it, such as RRR-d- α -6-tocopheramine, are also novel, and again constitute an aspect of this invention.

Example 3

The Synthesis of a Representative Benzopyran Derivative:

6-Folyltocopheramine

15 In this example, the synthesis of a representative benzopyran derivative of the invention, a 6-folyltocopheramine, is described.

The folate derivative of 6-tocopheramine is synthesized as follows. 6-Tocopheramine is synthesized by methods described in the literature, or by deoxygenation of tocopherol followed by nitration and reduction as described in Example
20 2. Folic acid is protected at the α -carboxyl with an O-benzyl blocking group and at the amines with Fmoc. The mixed anhydride is then prepared and reacted with 6-tocopheramine as described in Example 1. The chemical structure of 6-folyltocopheramine is shown in FIGURE 8. In FIGURE 8, the folyl-derivative structure described here (dotted lines) is shown overlaid on the synthetic model structure (solid
25 lines) from Example 1.

In FIGURE 8, R₁, R₂, R₃ and T₁ are methyl and T₂ is phytyl. For clarity the glutamyl carboxyl is still shown as blocked. The di, tri- or polyglutamyl folate amide can be made with minor modifications to the reaction. The carboxyl can be deprotected and cleavage of the O-benzyl ester will not result in hydrolysis of the peptide bond of the
30 conjugate to the tocopheramine, a clear advantage over the prior art. Note also that the synthesis effectively rules out the potential problem of steric hindrance from the vicinal methyl groups at the 5 and 7 positions on the 1-benzopyran ring. Furthermore, the folate derivative is γ -linked in its preferred form as a ligand for biological activity of the

derivative. The product is tocophilic and may be used in the manufacture of particulates, emulsions, nanoemulsions, microemulsions, or liposomes, or other multiphasic systems. Furthermore, by insertion of a suitable spacer, for example a polyethylene glycol or polyhydroxybutyrate of about one to two thousand molecular weight, between the folate
5 glutamyl moiety and the aminobenzopyran, a surfactant can be manufactured that displays the folate at an effective distance from the lipophilic tail to permit "targeting" of cancer cells.

Example 4

The Synthesis of a Representative Benzopyran Derivative:

10 6-Folyltocopheramine Monoglutamate

In this example, the synthesis of a representative benzopyran derivative of the invention, a 6-folyltocopheramine monoglutamate, is described.

The synthesis of the tocopheramine-folyl-monoglutamate conjugate was carried out via the dicyclohexylcarbodiimide coupling (DCC) method. 1.0 equivalent solution of
15 folic acid was activated by adding 1.2 equivalents of N-hydroxysuccinamide (NHS) followed by addition of 1.2 equivalents of dicyclohexyl carbodiimide (DCC) with stirring in the same sequence in dimethyl sulfoxide (DMSO) solution at 20°C. The resulting solution was stirred for 5 hours at 20°C and left in the refrigerator overnight. The organic mixture was filtered to remove dicyclohexyl urea and to the filtrate was added
20 1:3 acetone:ether to precipitate NHS-folate. The NHS-folate precipitate was washed with 6x100ml 1:3 acetone:ether and 2x 100ml isopropanol followed by vacuum drying at 30°C. To 1.00 equivalent solution of tocopheramine in tetrahydrofuran (THF) at 10°C was added NHS-folate solution dropwise with stirring and left overnight. The resulting solution was concentrated in vacuo and a yellowish brown oily liquid was obtained. The
25 concentrated organic liquid was dissolved in dichloromethane followed by a base wash using 3x 0.1N NaOH, 2 xNaHCO₃, 1x NaCl (satd.) and final drying over MgSO₄. The resulting solution was dried in vacuo to afford a yellowish brown oily product, yield 98%. FTIR of acid, amide, aliphatic hydrogen (C=O, C=O, C-H) at 1742, 1666, 2932, and 2862 cm⁻¹, respectively.

Example 5

The Synthesis of a Representative Benzopyran Derivative:

6-Methotrexyltocopheramine

In this example, the synthesis of a representative benzopyran derivative of the invention, a 6-methotrexyltocopheramine, is described.

The methotrexate derivative of 6-tocopheramine, a lipophilic drug analog with novel therapeutic properties, is synthesized as follows. 6-Tocopheramine is synthesized as described in Example 2. Folic acid is protected at the α -carboxyl with an O-benzyl blocking group, and at the amines with Fmoc. The mixed anhydride is then prepared and reacted with tocopheramine as described in Example 1. The chemical structure of 6-folyltocopheramine is shown in FIGURE 9. In FIGURE 9, the described structure (dotted lines) is shown overlaid on the actual structure (solid lines) from Example 1. In FIGURE 9, R₁, R₂, R₃ and T₁, are methyl, and T₂ is phetyl. For clarity the glutamyl carboxyl is still shown as blocked. The carboxyl can be deprotected and cleavage of the O-benzyl ester will not result in hydrolysis of the peptide bond of the conjugate to the tocopheramine, a clear advantage over the prior art. The product derivative is γ -linked to tocopheramine and serves as a prodrug for slow release of methotrexate following injection or ingestion. For increased bioavailability and accelerated sustained release of the methotrexate, the linkage can be made with a di, tri or polyglutamate. This example illustrates the synthesis of a tocan soluble prodrug such as may be incorporated in the manufacture of tocan-based particulates, emulsions, nanoemulsions, microemulsions, liposomes, or other multiphasic systems as described herein for parenteral or for oral delivery.

Example 6

The Synthesis of a Representative Benzopyran Derivative:

Tocopheramine Monoglutamate

In this example, the synthesis of a representative benzopyran derivative of the invention, tocopheramine monoglutamate, is described.

The synthesis of tocopheramine monoglutamate was carried out via mixed anhydride chemistry. 1.00 equivalent of N-t-BOC-L-glutamic acid γ -benzyl ester was activated by adding 1.00 equivalents of isobutyl chloroformate (IBCF) and N-methylmorpholine (NMM) in 100 ml of anhydrous tetrahydrofuran (THF) medium at -5°C. The reaction mixture was stirred at -5°C for 60 mins. The mixed anhydride was

filtered to remove the N-methylmorpholine hydrochloride salt (NNM:HCl). The filtrate was added dropwise to 1.00 equivalent solution of tocopheramine in THF containing 1.2 equivalents of triethylamine (TEA) at -5°C. The solution was left stirring overnight. After completion of the reaction, the THF was removed in vacuum and yellowish brown oil was obtained. The product was dissolved in dichloromethane (DCM) and washed with 2x 0.1N HCl, 2X NaHCO₃ (satd.), 2x NaCl (satd.). The resulting organic mixture was dried over MgSO₄ and dried in a vacuo to yield a yellowish brown oily product, yield 94%.

The deprotection of tocopheramine-(γ -benzyl) N-t-BOC-L-monoglutamate was carried out in two steps; first step being hydrogenation using Pd/C (10%) as catalyst (FTIR of amide, acid, amide, aliphatic hydrogen (C=O, C=O, C-H) are 1712 cm⁻¹ overlap C=O amide, 2930, and 2950 cm⁻¹, respectively. The second step is removal of N-t-BOC by dissolving tocopheramine-N-t-BOC-monoglutamate in 1:1 TFA:DCM and allowing the reaction mixture to stir for 2h at room temperature. The resulting solution was concentrated in vacuo followed by a base wash using 2x 0.1N NaOH, 2x NaHCO₃, 1x NaCl (satd.) and final drying over MgSO₄. The resulting solution was dried in vacuo to afford a yellowish brown oily product, yield 93%. FTIR: acid, amide, aliphatic (C=O, C=O, C-H) at 1712, 1666, 2930, and 2952 cm⁻¹, respectively.

Example 7

Physical Properties of Representative Tocopheramine Surfactants

Tocopheramine monoglutamate prepared as described in Example 4 was tested for its properties as a surfactant by measuring surface tension at a standard concentration in comparison to other surfactants. The surface tension (γ_s) of a 0.1% solution in 20 mM phosphate buffer pH 7.4 was measured using a K12 Tensiometer (Kruss, Charlotte NC) equipped with a Wilhelmy platinum plate.

Sample	Surface Tension (0.1 % w/v, dyne/cm)
Water	71.9
Tocopheramine monoglutamate	33.7
TPGS	34.5
Amisoft	24.8

For comparison, a purified non-ionic surfactant, TPGS (Eastman, Kingsport TN), and a pure anionic surfactant, Amisoft (Ajinomoto, Tokyo JP), were also tested at 0.1% in water. The tocopheramine monoglutamate derivative was comparable to TPGS, a nonionic surfactant sold commercially.

Example 8

The Synthesis of a Representative Benzopyran Derivative:

Tocopheramine Tetraglutamate

In this example, the synthesis of a representative benzopyran derivative of the invention, tocopheramine tetraglutamate, is described.

RRR-d- α -Tocopheramine is synthesized as described in Example 2. α -Glutamic acid tetrapeptide (blocked at the γ -carboxyls) is synthesized from the dipeptide. The tetrapeptide is conjugated to the 6-tocopheramine and deprotected to the product shown in FIGURE 10.

The γ -glutamyl analog is synthesized by a similar route. Note the acid-resistant, sterically hindered peptide bond proximate to the 6-benzopyranamine position. Similar compounds with the amine at the 5, 7, or 8 position of the appropriate benzopyran are also provided.

Example 9

The Synthesis of a Representative Benzopyran Derivative:

Tocopheramine Tetraglycine

In this example, the synthesis of a representative benzopyran derivative of the invention, tocopheramine tetraglycine, is described.

The synthesis of tocopheramine monoglycine was carried out via mixed anhydride chemistry. 1.00 equivalent of N-CBZ-Gly-Gly-Gly was activated by adding 1.00 equivalents of isobutyl chloroformate (IBCF) and N-methylmorpholine (NMM) in 100 ml of anhydrous tetrahydrofuran (THF) medium at -5°C. The reaction mixture was stirred at -5°C for 60 mins. The mixed anhydride was filtered to remove the N-methylmorpholine hydrochloride salt (NMM:HC1). The filtrate was added dropwise to 1.00 equivalent solution of tocopheramine in THF containing 1.2 equivalents of triethylamine (TEA) at -5°C. The solution was left stirring overnight. After completion of the reaction, the THF was removed in vacuum and yellowish oil was obtained. The product was dissolved in dichloromethane (DCM) and washed with 2x 0.1N HCl, 2X NaHCO₃ (satd.), 2x NaCl (satd.). The resulting organic mixture was dried over MgSO₄ and dried in a vacuo to yield a yellowish brown oily product. The deprotection of N-CBZ-Gly-Gly-Gly-Gly-tocopheramine was carried out in methanol by hydrogenation using Pd/C (10%) as catalyst with constant stirring overnight to afford tocopheramine tetraglycine.

Similar compounds can be made with a variety of hydrophilic substituents as disclosed in the specification.

Example 10

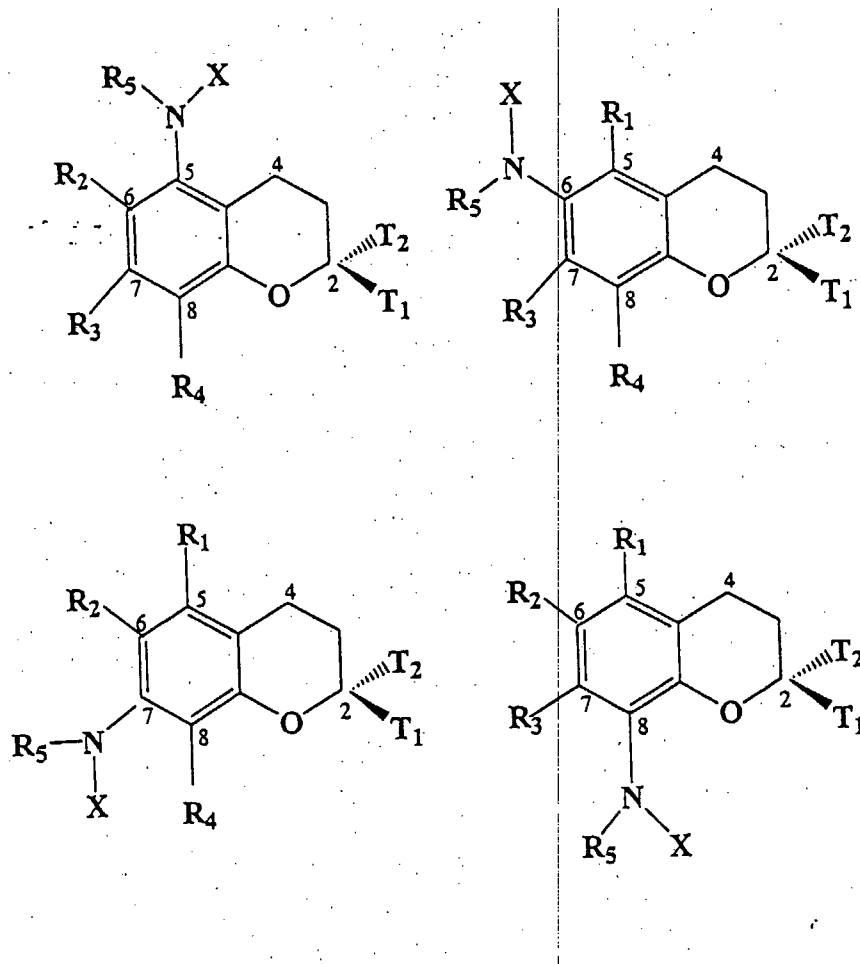
Oral Bioavailability

The benzopyran derivatives of the invention can be evaluated as bioavailability enhancers in an in vitro CACO-2 tissue culture model.

While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A compound having the structure:



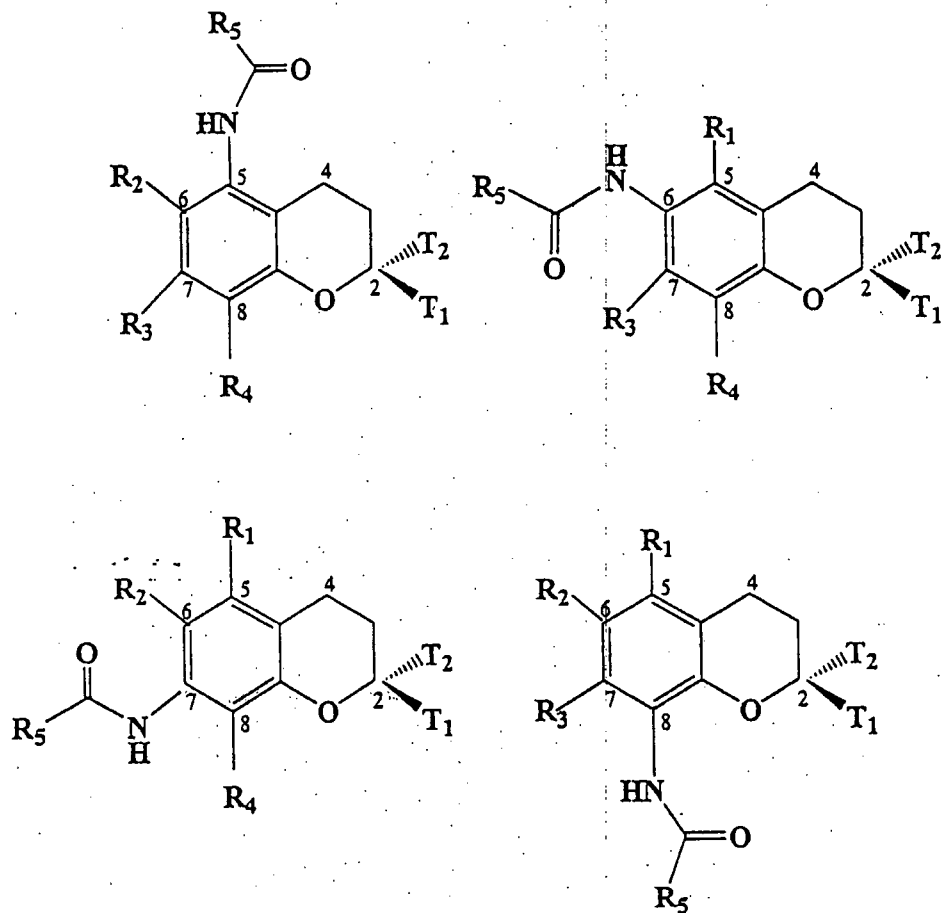
wherein R₁, R₂, R₃, and R₄ are selected from the group consisting of hydrogen, C1-C4 alkyl, carboxyl, and hydroxyl;

wherein R₅ is a hydrophilic group that renders the compound no more than weakly bonded to a C8 column packing;

wherein T₁ and T₂ are selected from the group consisting of hydrogen, methyl, ethyl, isoprenyl, terpenyl, phytlyl, and trienyl; and

wherein X is selected from the group consisting of hydrogen, methyl, and ethyl.

2. A compound having the structure:



wherein R₁, R₂, R₃, and R₄ are selected from the group consisting of hydrogen, C1-C4 alkyl, carboxyl, and hydroxyl;

wherein R₅ is a hydrophilic group that renders the compound no more than weakly bonded to a C8 column packing; and

wherein T₁ and T₂ are selected from the group consisting of hydrogen, methyl, ethyl, isoprenyl, terpenyl, phytanyl, and trienyl.

3. The compound of Claim 2, wherein R₅CO- comprises an amino acid residue.

4. The compound of Claim 2, wherein R₅CO- comprises a glutamate residue.

5. The compound of Claim 2, wherein R5CO- comprises a tetraglutamate residue.
6. The compound of Claim 2, wherein R5CO- comprises a glycine residue.
7. The compound of Claim 2, wherein R5CO- comprises a tetraglycine residue.
8. The compound of Claim 2, wherein R5CO- comprises a folate group.
9. The compound of Claim 2, wherein R5CO- comprises a methotrexate group.
10. RRR-d- α -6-aminotocopherol.
11. d- δ -5-Aminotocopherol.
12. d- δ -7-Aminotocopherol.
13. d- δ -5-Aminotocotrienol.
14. d- δ -7-Aminotocotrienol.
15. 6-Folyltocopheramine.
16. 6-Folyltocopheramine monoglutamate.
17. 6-Methotrexyltocopheramine.
18. Tocopheramine monoglutamate.
19. Tocopheramine tetraglutamate.
20. Tocopheramine tetraglycine.

1/10

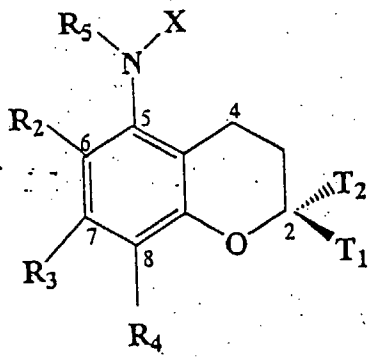


FIG. 1A

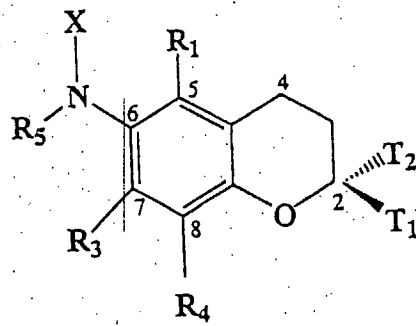


FIG. 1B

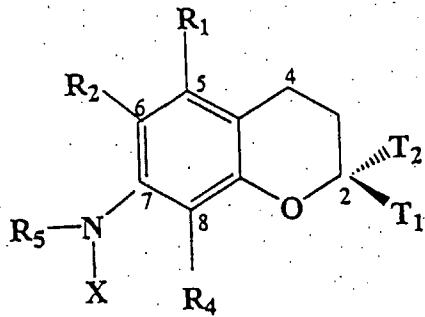


FIG. 1C

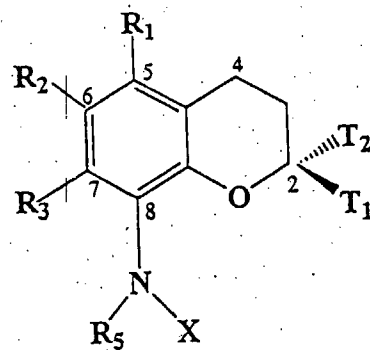


FIG. 1D

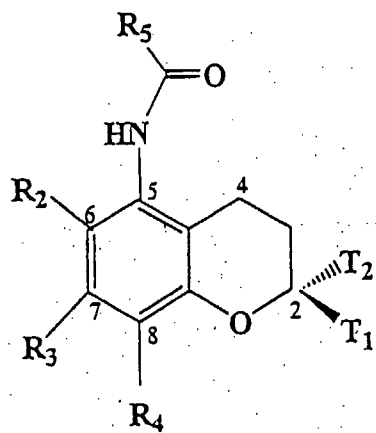


FIG. 2A

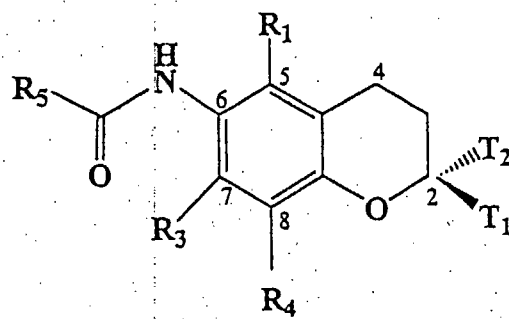


FIG. 2B

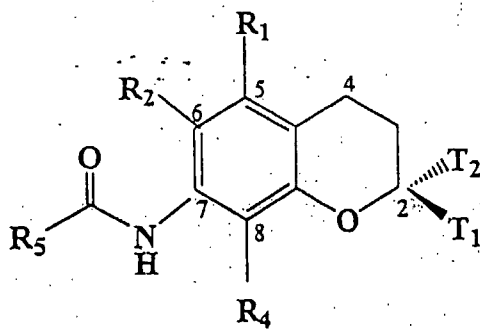


FIG. 2C

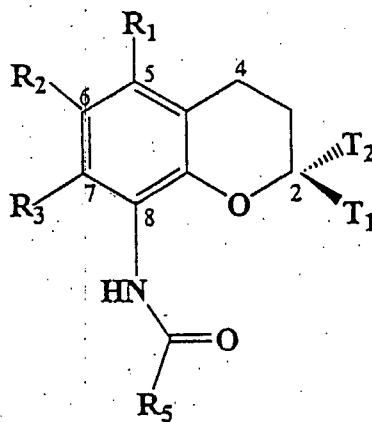


FIG. 2D

3/10

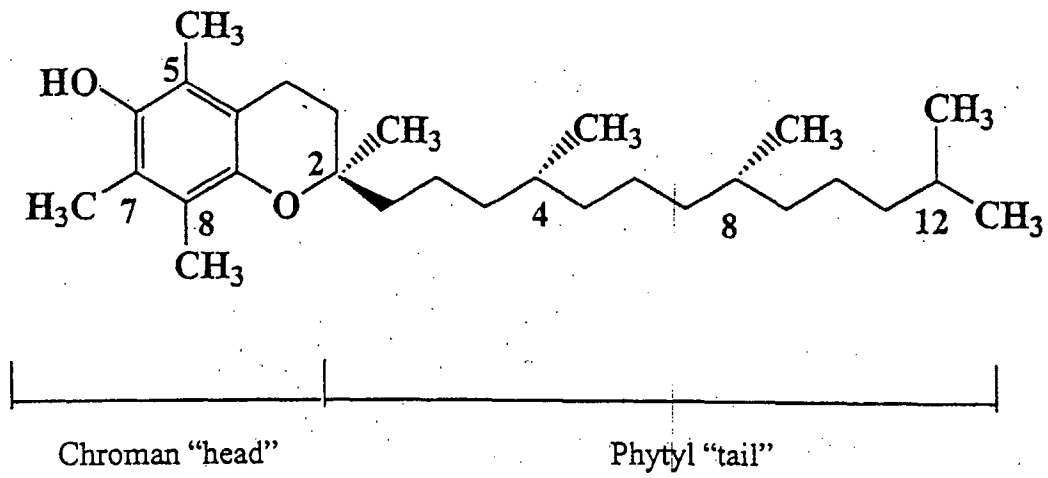


FIG. 3

4/10

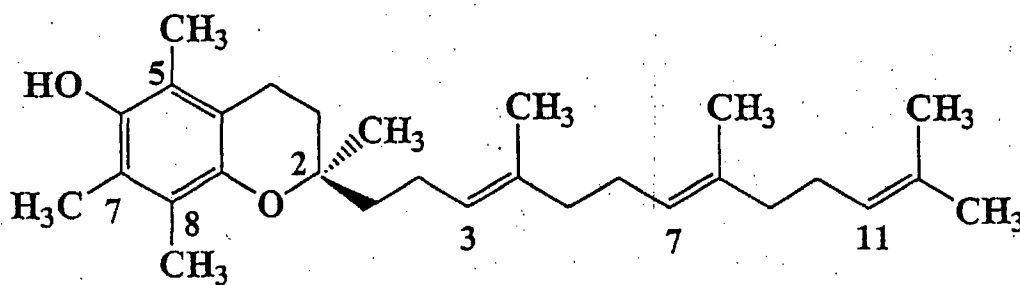


FIG. 4

5/10

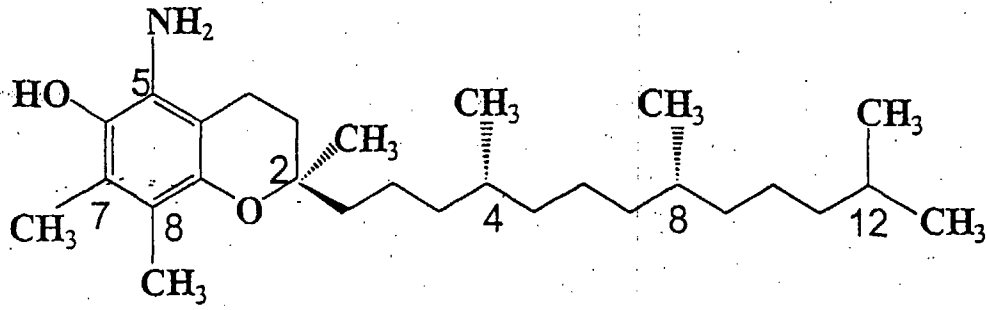


FIG. 5

7/10

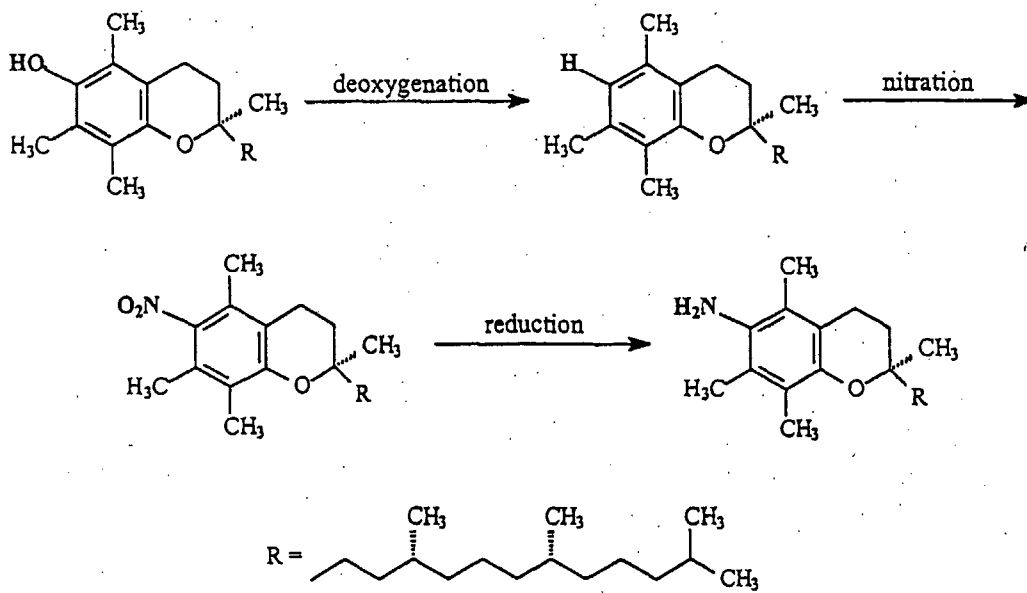


FIG. 7

8/10

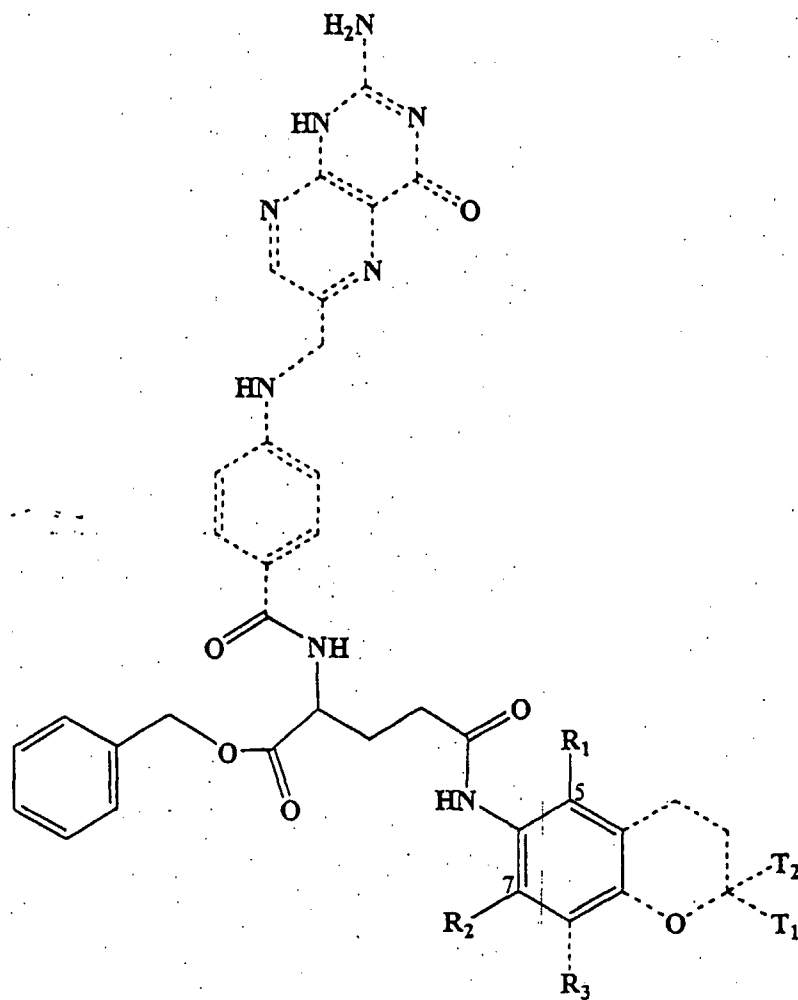


FIG. 8

9/10

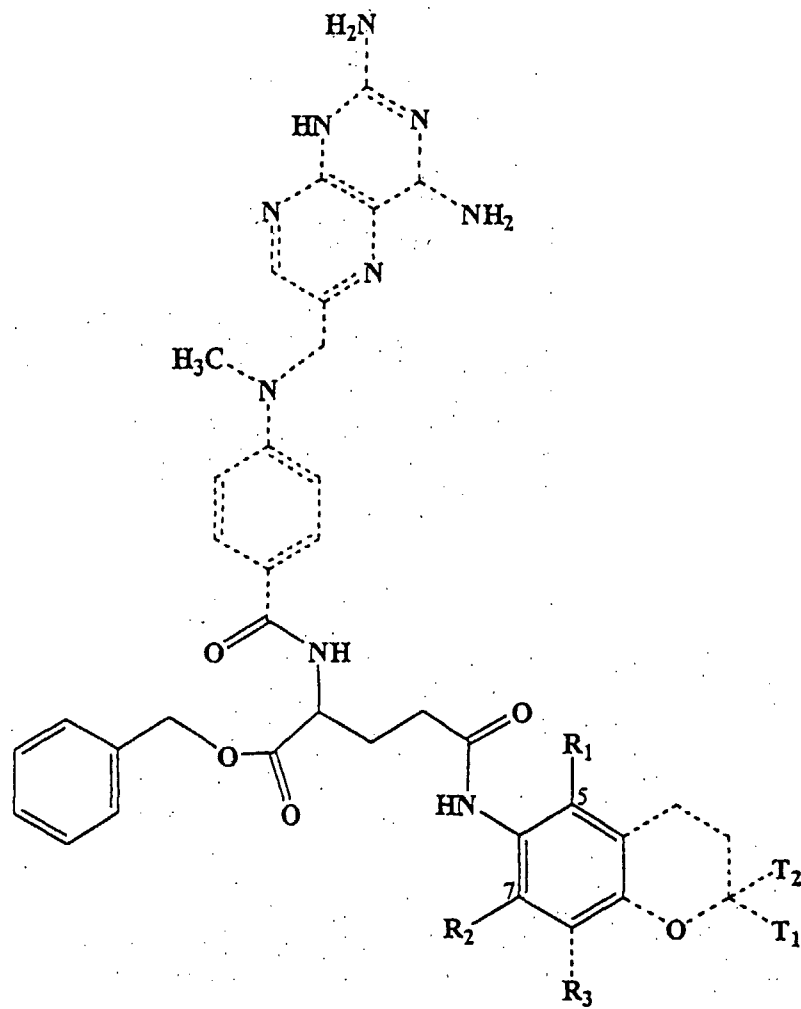


FIG. 9

10/10

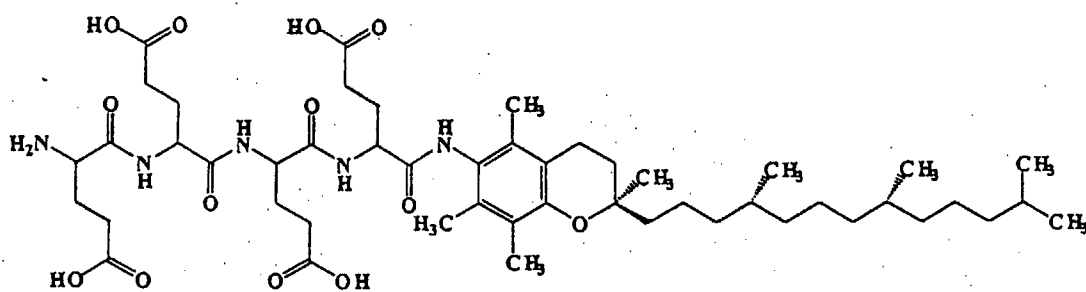


FIG. 10