ECHINOCANDIN PHARMACEUTICAL FORMULATIONS CONTAINING MICELLE-FORMING SURFACTANTS

FIELD OF THE INVENTION

The present invention relates to pharmaceutical formulations containing an echinocandin compound, in particular, the incorporation of micelle-forming surfactants to enhance stability and water solubility.

BACKGROUND OF THE INVENTION

Parenteral (ip) formulations of pharmaceutical drugs may be administered to patients via intramuscular (im), intravenous (iv) or subcutaneous methodology. The formulation that is developed for a particular drug is dependent on a variety of issues. For example, it is well-known in the art that a formulation should be water-soluble and stable. If freeze-dried the formulation should be capable of forming a well-formed cake and readily reconstitutable (usually in less than one minute). Finally, the formulation should have an acceptable appearance and be prepared from generally accepted, safe excipients.

Stability is an important consideration when designing a formulation, especially for ip applications. For practical reasons, it must be possible to store the formulation for at least two years. Therefore, it is often desirable to freeze dry the formulation to achieve better shelf-life and storage at room temperature.

The instability and poor water solubility (<0.1 mg/ml) of the echinocandin compounds make them particularly difficult to formulate. Most of the formulations tested to date have a shelf life of less than one year. Generally, a shelf life of two or more years is a required. Consequently, a formulation containing an echinocandin compound may require freeze-drying to achieve the necessary stability.

The poor water solubility of the echinocandin compounds affords a further challenge in formulating an ip formulation containing echinocandin active materials. One method of formulating such compounds is by the addition of a surfactant which enhances the solubility of the drug. However, it is generally well-known in the art that the use of a surfactant above a certain concentration generally limits the ability to freeze dry the formulation. A typical freeze-dried formulation has a surfactant concentration less than 5% by weight. According to a market survey of freeze-dried pharmaceutical formulations containing surfactants, the surfactant concentration is usually less than 5% weight in the freeze dried product. See, Carpenter et al., Pharm. Res., 14 (8), 969-975, 1977 to 1997, Physicians' Desk Reference, 50th edition, Medical Economics, Co. NJ (1996). It is generally believed that a formulation with higher concentrations of surfactant is not likely to form a freeze-dried product with desirable characteristics. Specifically, the presence of the surfactant causes the freeze-dried cake to "collapse" resulting in a residue at the bottom of the vial instead of a well-formed cake. The residue is generally less stable, difficult to reconstitute, and non-reproducible.

Because of the poor water solubility of the echinocandin compounds, generally 2-4% (weight by volume) of a surfactant is required to obtain an acceptable concentration of the echinocandin compound in solution. As discussed above, freeze-drying is hindered at this level of surfactant. Therefore, there is a need for a formulation that improves the solubility of echinocandin compounds in water yet allows freeze drying to obtain optimum stability.

SUMMARY OF THE INVENTION

Applicants have discovered a group of surfactants that solubilize echinocandin compounds at higher concentrations and surprisingly retain the ability to freeze dry the formulation. In one embodiment of the present invention, a parenteral pharmaceutical formulation is provided that comprises (i) an echinocandin compound (or a pharmaceutically acceptable salt thereof), (ii) a pharmaceutically acceptable, micelle-forming surfactant (e.g., polysorbates, lecithin, bile salts, polyoxyethylene castor oils and mixtures thereof), and (iii) a non-toxic, aqueous solvent. The pharmaceutical solution

formulation may optionally contain one or more of a stabilizing agent, a tonicity agent and/or a buffer. The weight ratio of echinocandin to surfactant is from about 1:1.75 to about 1:25 (more preferably in a ratio of about 1:2 to about 1:3) and the echinocandin compound is present in an amount greater than or equal to 1 mg/ml. The surfactant is generally present in an amount greater than 1% weight per volume.

In one embodiment of the present invention, a freeze-dried pharmaceutical formulation is provided that comprises (i) an echinocandin compound (or a pharmaceutically acceptable salt thereof), (ii) a pharmaceutically acceptable, micelle-forming surfactant (e.g., polysorbates, lecithin, bile salts, polyoxyethylene castor oils, and mixtures thereof), and (iii) a bulking agent. The micelle-forming surfactant is present in an amount greater than 5% by weight in the freeze dried product and the ratio of echinocandin to surfactant is from about 1:1.75 to about 1:25 (preferably in a ratio of about 1:2 to about 1:3). The pharmaceutical freeze-dried formulation may optionally contain one or more of a stabilizing agent and/or a buffer. A parenteral pharmaceutical formulation prepared from the freeze-dried formulation is also provided.

In yet another embodiment of the present invention, a process is provided for preparing a parenteral formulation, which comprises mixing an echinocandin compound (or echinocandin/carbohydrate complex) and a pharmaceutically acceptable, micelle-forming surfactant in an aqueous solvent.

In another embodiment of the present invention, a process is provided for making a freeze-dried formulation comprising in the following order the steps of: (i) dissolving into an aqueous solvent an echinocandin compound (or echinocandin/carbohydrate complex) in the presence of a pharmaceutically acceptable, micelle-forming surfactant, wherein the surfactant is present in an amount greater than 1% weight by volume; (ii) sterile filtering the solution; and (iii) freeze-drying the solution. Generally, a bulking agent is added before freeze-drying the solution. Optionally, one may add one or more buffers, stabilizing agents, tonicity agents, or combinations thereof before step (iii).

An alternative preparation for a freeze-dried formulation is also provided which comprises (i) buffering a non-toxic aqueous solvent to a pH between 4.0 and 5.0 to form a buffered solution; (ii) adding to the buffered solution a pharmaceutically acceptable, micelle-forming surfactant; (iii) cooling the solution from step (ii) to a temperature between

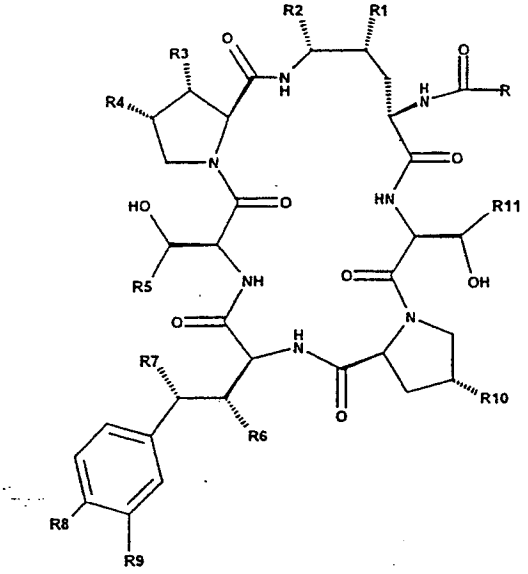
5° and 15°C (preferably between 7° and 10°C) to form a cooled solution; (iv) adding a slurry comprising an echinocandin compound or echinocandin/carbohydrate complex and a second non-toxic aqueous solvent to the cooled solution; (v) sterile filtering said solution from step (iv); and (vi) freeze-drying said solution from step (v). One or more bulking agents, stabilizing agents and/or tonicity agents may optionally be added prior to step (v).

In another embodiment of the present invention, a parenteral pharmaceutical product is provided which is prepared by dissolving into an aqueous solvent an echinocandin compound (or echinocandin/carbohydrate complex) in the presence of a pharmaceutically acceptable, micelle-forming surfactant to form a solution, wherein the surfactant is present in an amount greater than 1% weight per volume; (ii) sterile filtering the solution; and (iii) freeze-drying the solution in a vial, wherein the weight ratio of echinocandin to surfactant is from about 1:1.75 to about 1:25. When ready for use, a non-toxic, aqueous solvent is added to the vial.

In yet another embodiment of the present invention, a method is provided for treating an antifungal infection in a mammal in need thereof, which comprises administering to the mammal the parenteral formulation described above or a parenteral formulation prepared by adding a pharmaceutically acceptable aqueous solvent to one of the freeze-dried formulations described above.

Amounts and percentages are described herein as weight units unless otherwise stated.

The term "echinocandin" refers to a compound having the following general structure:



where: R is an alkyl group, an alkenyl group, an alkynyl group, an aryl group, heteroaryl group, or combinations thereof; R_1, R_2, R_3, R_6, R_7, and R_{10} are independently hydroxy or hydrogen; R_4 is hydrogen, methyl or $-CH_2C(O)NH_2$; R_5 and R_{11} are independently methyl or hydrogen; R_8 is -OH, $-OPO_3H_2$, $-OPO_3HCH_3$, $-OPO_2HCH_3$, or $-OSO_3H$; and R_9 is -H, -OH, or $-OSO_3H$.

The term "alkyl" refers to a hydrocarbon radical of the general formula C_nH_{2n+1} containing from 1 to 30 carbon atoms unless otherwise indicated. The alkane radical may be straight, branched, cyclic, or multi-cyclic. The alkane radical may be substituted or unsubstituted. Similarly, the alkyl portion of an alkoxy group or alkanoate have the same definition as above.

The term "alkenyl" refers to an acyclic hydrocarbon containing at least one carbon-carbon double bond. The alkene radical may be straight, branched, cyclic, or multi-cyclic. The alkene radical may be substituted or unsubstituted.

The term "alkynyl" refers to an acyclic hydrocarbon containing at least one carbon carbon triple bond. The alkyne radical may be straight, or branched. The alkyne radical may be substituted or unsubstituted.

The term "aryl" refers to aromatic moieties having single (e.g., phenyl) or fused ring systems (e.g., naphthalene, anthracene, phenanthrene, etc.). The aryl groups may be substituted or unsubstituted. Substituted aryl groups include a chain of aromatic moieties (e.g., biphenyl, terphenyl, phenylnaphthalyl, etc.).

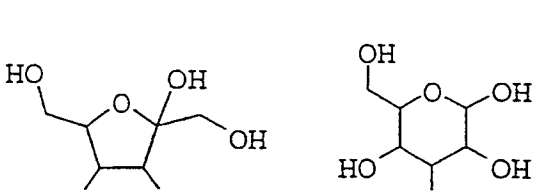
The term "heteroaryl" refers to aromatic moieties containing at least one heteroatom within the aromatic ring system (e.g., pyrrole, pyridine, indole, thiophene, furan, benzofuran, imidazole, pyrimidine, purine, benzimidazole, quinoline, etc.). The aromatic moiety may consist of a single or fused ring system. The heteroaryl groups may be substituted or unsubstituted.

Within the field of organic chemistry and particularly within the field of organic biochemistry, it is widely understood that significant substitution of compounds is tolerated or even useful. In the present invention, for example, the term alkyl group allows for substituents which is a classic alkyl, such as methyl, ethyl, isopropyl, isobutyl, tertiary butyl, hexyl, isooctyl, dodecyl, stearyl, etc. The term group specifically envisions and allows for substitutions on alkyls which are common in the art, such as hydroxy, halogen, alkoxy, carbonyl, keto, ester, carbamato, etc., as well as including the unsubstituted alkyl moiety. However, it is generally understood by those skilled in the art that the substituents should be selected so as to not adversely affect the pharmacological characteristics of the compound or adversely interfere with the use of the medicament. Suitable substituents for any of the groups defined above include alkyl, alkenyl, alkynyl, aryl, halo, hydroxy, alkoxy, aryloxy, mercapto, alkylthio, arylthio, mono- and di-alkyl amino, quaternary ammonium salts, aminoalkoxy, hydroxyalkylamino, aminoalkylthio, carbamyl, carbonyl, carboxy, glycolyl, glycyl, hydrazino, guanyl, and combinations thereof.

"Echinocandin/carbohydrate complex" refers to a crystalline complex formed between an echinocandin compound and an carbohydrate when the echinocandin is recrystallized from a solvent in the presence of the carbohydrate. A more detailed description of the echinocandin/carbohydrate complexes may be found in Larew, et al., filed on March 3, 1999 entitled "Echinocandin/Carbohydrate Complexes."

"Carbohydrate" refers to an aldehydic or ketonic derivative of polyhydric alcohols represented by the formulas $C_n(H_2O)_n$ (e.g., glucose, $C_6(H_2O)_6$; sucrose, $C_{12}(H_2O)_{11}$). Carbohydrates include compounds with relatively small molecules, such as the simple sugars (e.g., monosaccharides, disaccharides, etc.), as well as macromolecular (polymeric) substances such as starch, glycogen, and cellulose polysaccharides. Sugars are carbohydrates (saccharides) having the general composition $(CH_2O)_n$ and simple derivatives thereof. Although the simple monomeric sugars (glycoses) are described as polyhydroxy

aldehydes or ketones, e.g., $HOCH_2–(CHOH)_4–CHO$ for aldohexoses (e.g., glucose) or $HOCH_2–(CHOH)_3–CO–CH_2OH$ for 2-ketoses (e.g., fructose), the structures are commonly written as five (furanose) or six (pyranose) membered ring cyclic ethers, e.g.



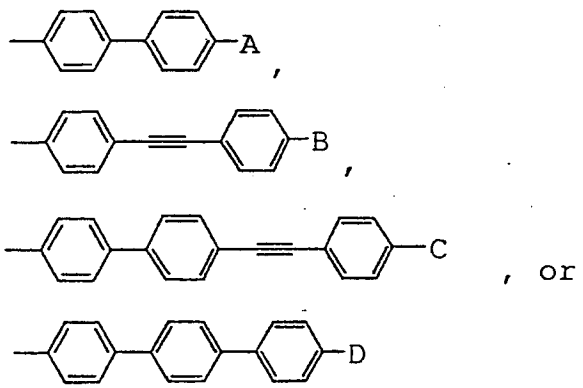
The term "micelle-forming surfactant" refers to an amphiphilic material that spontaneously and reversibly forms a water soluble aggregate. For a more detailed description of micelle formation and a listing of micelle-forming surfactants see Attwood et al. Surfactant Systems, Their Chemistry, Pharmacy and Biology, Chapman and Hall (1983). Block copolymers of propylene oxide and ethylene oxide do not perform well in the formulations of the present invention; therefore, these block copolymers are not considered within the meaning of micelle-forming surfactants.

The term "pharmaceutically acceptable" when used herein as an adjective, means substantially non-toxic and substantially non-deleterious to the recipient.

DETAILED DESCRIPTION

The cyclic peptides used in the present invention may be produced by culturing various microorganisms. Suitable natural product starting materials belonging to the echinocandin cyclic peptide family include Echinocandin B, Echinocandin C, Echinocandin D, Aculeacin Aγ, Mulundocandin, Sporiofungin A, Pneumocandin A_0, WF11899A, and Pneumocandin B_0. In general, the cyclic peptides may be characterized as a cyclic hexapeptide nucleus with an acylated amino group on one of the amino acids. The amino group on the naturally-occurring cyclic peptide is typically acylated with a fatty acid group forming a side chain off the nucleus. Examples of naturally-occurring acyl groups include linoleoyl (Echinocandin B, C and D), palmitoyl (Aculeacin Aγ and WF11899A), stearoyl, 12-methylmyristoyl (Mulundocandin), 10,12-dimethylmyristoyl (Sporiofungin A and Pneumocandin A_0) and the like.

Semi-synthetic derivatives may be prepared by removing the fatty acid side chain from the cyclic peptide nucleus to produce a free amino group (i.e., no pendant acyl group - C(O)R). The free amine is then reacylated with a suitable acyl group. For example, the echinocandin B nucleus has been re-acylated with certain non-naturally occurring side chain moieties to provide a number of antifungal agents. See, i.e., U.S. Patent No. 4,293,489. Those skilled in the art will appreciate that the N-acyl side chain encompasses a variety of side chain moieties known in the art. Suitable side chain moieties include substituted and unsubstituted alkyl groups, alkenyl groups, alkynyl groups, aryl groups, heteroaryl groups and combinations thereof. Preferably, the side chain contains both a linearly rigid section and a flexible alkyl section to maximize antifungal potency. Representative examples of preferred acyl side chains include R groups having the following structures:



, or

where A, B, C and D are independently hydrogen, C_1-C_{12} alkyl, C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, C_1-C_{12} alkylthio, halo, or -O-$(CH_2)_m$-[O-$(CH_2)_n$]$_p$-O-(C_1-C_{12} alkyl), or -O-$(CH_2)_q$-X-E; m is 2, 3 or 4; n is 2, 3 or 4; p is 0 or 1; q is 2, 3 or 4; X is pyrrolidino, piperidino or piperazino; and E is hydrogen, C1-C12 alkyl, C3-C12 cycloalkyl, benzyl or C3-C12 cycloalkylmethyl.

As noted above, the cyclic peptides described herein may be prepared by fermentation of known microorganisms as described in the art. The subsequent deacylation is typically carried out enzymatically using a deacylase enzyme by known materials and procedures described in the art.

For example, U.S. Patent No. 3,293,482 describes the deacylation and preparation of the cyclic peptide of formula I where R_4, R_5, and R_{11} are methyl, R_9 is hydrogen, and R_1, R_2, R_3, R_6, R_7, R_8 and R_{10} are each hydroxy. U.S. Patent No. 4,299,763 describes the deacylation and preparation of the cyclic peptide of formula I where R_4, R_5, and R_{11} are methyl, R_2 is hydroxy, R_7 and R_9 are hydrogen and R_1, R_3, R_6, R_8 and R_{10} are each hydroxy. U.S. Patent No. 3,978,210 describes the preparation of aculeacin. U.S. Patent No. 4,304,716 describes the deacylation and preparation of the cyclic peptide of formula I where R_5 is $-CH_2C(O)NH_2$; R_{11} is methyl; R_4 and R_9 are hydrogen; R_1, R_2, R_3, R_6, R_7, R_8 and R_{10} are each hydroxy and the acyl group with substituent R is myristoyl.

Cyclic peptides where R_2 and R_7 are each hydrogen may be prepared by subjecting the corresponding compound (where R_2 and R_7 are each hydroxy; the ornithine alpha-amino group may be a free amino group or acylated) to a strong acid and a reducing agent at a temperature of between -5°C and 70°C, in a suitable solvent. Suitable strong acids include trichloroacetic acid, trifluoroacetic acid or boron trifluoride etherate. A preferred strong acid is trifluoroacetic acid. Suitable reducing agents include sodium cyanoborohydride or triethylsilane. A preferred reducing agent is triethylsilane. Suitable solvents include methylene chloride, chloroform or acetic acid, preferably methylene chloride. The strong acid is present in an amount from about 2 to 60 mol per mol of substrate, and the reducing agent is present in an amount from about 2 to 60 mol per mol of substrate. The acid reduction process selectively removes the aminal (R_2) and benzylic (R_7) hydroxy groups.

Acylation of the α-amino group on the ornithine unit may be accomplished in a variety of ways well known to those skilled in the art. For example, the amino group may be acylated by reaction with an appropriately substituted acyl halide, preferably in the presence of an acid scavenger such as a tertiary amine (e.g., triethylamine). The reaction is typically carried out at a temperature between about -20°C to 25°C. Suitable reaction solvents include polar aprotic solvents, such as dioxane or dimethylformamide. Solvent choice is not critical so long as the solvent employed is inert to the ongoing reaction and the reactants are sufficiently solubilized to effect the desired reaction.

The amino group may also be acylated by reaction with an appropriately substituted carboxylic acid, in the presence of a coupling agent. Suitable coupling agents include dicyclohexylcarbodiimide (DCC), N,N'-carbonyldiimidazole, bis(2-oxo-3-

oxazolidinyl)phosphinic chloride (BOP-Cl), N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), benzotriazole-1-yloxy- tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and the like.

Alternately, the amino group may be acylated with an activated ester of a carboxylic acid such as p-nitrophenyl, 2,4,5-trichlorophenyl, hydroxybenzotriazole hydrate ($HOBT \cdot H_2O$), pentafluorophenol, and N-hydroxysuccinimide carboxylate esters. Preferred acylating moieties are the 2,4,5-trichlorophenyl and HOBT carboxylate esters. The reaction is typically ran 1 to 65 hours at a temperature from about 0°C to 30°C in an aprotic solvent. The reaction is generally complete after about 24 to 48 hours when carried out at a temperature between about 15°C to 30°C. Suitable solvents include tetrahydrofuran and dimethylformamide or mixtures thereof. The amino group is generally present in equimolar proportions relative to the activated ester or with a slight excess of the amino group.

The R-COOH precursor acids are prepared by hydrolyzing a nitrile of the formula R-CN or an ester of the formula R-COO(C_1-C_4 alkyl). The nitrile and ester intermediates may be prepared using procedures known in the art. For example, the nitrile and ester intermediates where R is an alkoxy aryl moiety may be prepared using Procedure A or Procedure B.

Procedure A One equivalent of an alkyl bromide, iodide, or *p*-toluenesulfonate is added to a mixture containing one equivalent of a base, such as potassium *t*-butoxide or potassium carbonate (K_2CO_3), and one equivalent of an hydroxy aryl compound in 200-300ml of acetonitrile (CH_3CN). The reaction mixture is refluxed for 6h and then concentrated *in vacuo* to provide a residue which is dissolved in a Et_2O/2N NaOH mixture. The resulting layers are separated and the organic layer is dried over magnesium sulfate ($MgSO_4$), filtered and dried to provide the alkoxy aryl product.

Procedure B Diethylazodicarboxylate (1 equiv.) is added dropwise to a mixture containing an hydroxy aryl compound (1 equiv.), an alkyl alcohol (1 equiv.) and triphenylphosphine (1 equiv.) in 200-300ml of THF. After 17h, the solvent is removed *in vacuo* to provide a residue which is dissolved in Et_2O. The resulting mixture is washed with a 2N NaOH solution, dried over $MgSO_4$, filtered and concentrated to provide a product

which is then crystallized from a Et_2O/pentane mixture or, if the product contains a tertiary amine, the hydrochloride salt is formed and crystallized from a methanol (MeOH)/EtOAc mixture. The nitrile and ester intermediates where R is an alkynyl aryl moiety may be prepared using Procedure C.

Procedure C A mixture containing Et_2O (2 equiv.), palladium dichloride (0.05 equiv.), triphenylphosphine (0.1 equiv.), cuprous iodide (0.025 equiv.) and an alkyne (1 equiv.) is added to one equivalent of an aryl bromide, iodide, or trifluoromethanesulfonate in CH_3CN (600ml/0.1mol of aryl reactant), under nitrogen (N_2). The resulting mixture is refluxed for 17h and then the solvent is removed *in vacuo* to provide a residue which is slurried in 300 ml of Et2O and then filtered. The filtrate is washed with a 1N HCl solution, dried over $MgSO_4$, filtered and then dried to provide the product. The ester intermediates where R is a terphenyl moiety may be prepared using Procedure D.

Procedure D

1. *Formation of boronic acid reactant*

Butyl lithium (1.2 equivalents) is added to one equivalent of a cold (-78°C) aryl halide in THF. After 15 minutes, triisopropyl borate (2 equiv.) is added. After 10 minutes, the reaction mixture is warmed to room temperature and quenched by the addition of water (H_2O), followed by the addition of 1N HCl. The resulting layers are separated and the organic layer is concentrated *in vacuo* to provide a solid which is collected by filtration and washed with hexane.

2. *Formation of terphenyl ester*

Tetrakis(triphenylphosphine)palladium (0.03 equiv.) is added to a mixture containing an aryl boronic acid (1 equiv.), K_2CO_3 (1.5 equiv.) and methyl 4-iodobenzoate (1 equiv.) (or trichlorophenyl ester of iodobenzoate) in N_2-purged toluene. The reaction mixture is refluxed for 7h and then decanted to remove the K_2CO_3 and dried *in vacuo* to provide a residue. This residue is triturated in CH_3CN and filtered to provide the product. The aryl nitriles and esters described above may be converted to the corresponding carboxylic acids by hydrolysis using Procedure E or Procedure F.

Procedure E An aryl nitrile is dissolved in ethanol (EtOH) and an excess of 50% NaOH solution and refluxed for 2h. Water is added to the reaction mixture until a solid

precipitates. This solid is collected by filtration, added to a dioxane/6N HCl mixture and the resulting mixture is refluxed for 17h. When the reaction is substantially complete, the carboxylic acid product is crystallized by the addition of H_2O and then collected by filtration and dried *in vacuo*.

Procedure F An excess of 2N NaOH is added to an aryl ester in MeOH, and the resulting solution is refluxed for 5h and then acidified by the addition of excess HCl. Water is added to the reaction mixture until a solid (carboxylic acid) precipitates. The carboxylic acid is collected by filtration and dried *in vacuo*. The carboxylic acids may be converted to the corresponding 2,4,5-trichlorophenyl esters using Procedure G, below. The activated esters are then used to acylate the amino nucleus.

Procedure G A mixture containing an aryl carboxylic acid (1 equiv.), 2,4,5-trichlorophenol (1 equiv.) and DCC (1 equiv.) in CH_2Cl_2 is stirred for 17h and then filtered. The filtrate is concentrated to provide a residue which is dissolved in Et_2O, filtered, and then pentane is added until crystallization begins. The crystals are collected by filtration and dried *in vacuo*. Alternatively, the carboxylic acid may be activated by conversion to the corresponding hydroxybenzotriazole ester using Procedure H.

Procedure H An aryl carboxylic acid (1 equiv.) and a slight excess of N-mesylate substituted hydroxybenzotriazole (1.2 equiv.) were reacted in the presence of a slight excess of a base such as triethylamine (Et_3N) (1.3 equiv.) in DMF, under N_2. When the reaction was complete, the mixture was diluted with toluene and washed with H_2O. The organic portion was diluted with H_2O and then filtered using *t*-butyl methyl ether (MTBE) for transferring the material. The resultant solid was washed with MTBE and then dried *in vacuo*.

The echinocandin compound may be isolated and used per se or in the form of its pharmaceutically acceptable salt or hydrate, or as a echinocandin/carbohydrate complex. The term "pharmaceutically acceptable salt" refers to non-toxic acid addition salts derived from inorganic and organic acids. Suitable salt derivatives include halides, thiocyanates, sulfates, bisulfates, sulfites, bisulfites, arylsulfonates, alkylsulfates, phosphonates, monohydrogen-phosphates, dihydrogenphosphates, metaphosphates, pyrophosphonates, alkanoates, cycloalkylalkanoates, arylalkonates, adipates, alginates, aspartates, benzoates, fumarates, glucoheptanoates, glycerophosphates, lactates, maleates, nicotinates, oxalates,

palmitates, pectinates, picrates, pivalates, succinates, tartarates, citrates, camphorates, camphorsulfonates, digluconates, trifluoroacetates, and the like.

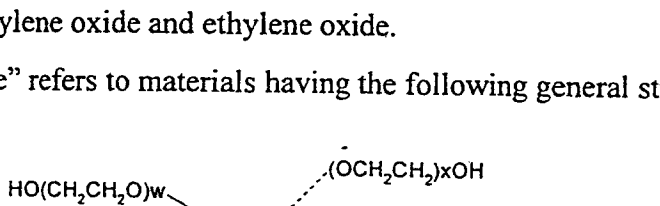
"Echinocandin/carbohydrate complex" refers to a crystalline complex formed between an echinocandin compound and an carbohydrate (or sugar) when the echinocandin is recrystallized from a solvent in the presence of the carbohydrate. A more detailed description of the echinocandin/carbohydrate complexes may be found in Larew, et al., filed on March 3, 1999 entitled "Echinocandin/Carbohydrate Complexes" and incorporated herein by reference. The complexes are formed using standard crystallization procedures such as those typically performed for purifying compounds by recrystallization. The echinocandin material and carbohydrate are dissolved at an elevated temperature (approximately 45 to 60°C, preferably less than 55°C) in a solvent. The solution is then slowly cooled until the crystallization begins. A seed crystal (such as a previously crystallized complex or an insoluble sugar) may be added to initiate crystallization. Suitable solvents include any solvent, or mixture of solvents, inert to the ongoing reaction that sufficiently solubilizes the reactants to afford a medium within which to effect the desired complexation between the carbohydrate and the echinocandin compound, such as protic or ketone solvents including methanol, ethanol, benzyl alcohol, as well as mixtures of benzyl alcohol with solvents such as methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol, 2-butanol, *t*-butanol, 2-pentanol, 2-methyl-1-propanol, MEK, acetone, ethyl acetate, toluene, acetonitrile, fluorobenzene, methylene chloride, nitromethane, or cyclic ketones such as cyclopentanone and cyclohexanone. Preferred solvents include methanol, ethanol, benzyl alcohol, and mixtures of benzyl alcohol with methyl ethyl ketone, ethyl acetate, and acetonitrile.

Suitable carbohydrates include adonitol, arabinose, arabitol, ascorbic acid, chitin, D-cellubiose, 2-deoxy-D-ribose, dulcitol, (S)-(+)-erythrulose, fructose, fucose, galactose, glucose, inositol, lactose, lactulose, lyxose, maltitol, maltose, maltotriose, mannitol, mannose, melezitose, melibiose, microcrystalline cellulose, palatinose, pentaerythritol, raffinose, rhamnose, ribose, sorbitol, sorbose, starch, sucrose, trehalose, xylitol, xylose and hydrates thereof. Suitable carbohydrates also include the D and L enantiomers, as well as the alpha and beta anomers of the compounds listed above. Preferred carbohydrates are the simple sugars (e.g., mono- and di-saccharides).

The echinocandin compound can be present in a formulation of the present invention prior to freeze drying at concentrations greater than or equal to 1 mg/ml. Generally, the echinocandin compound is present in a range from about 1 mg/ml to about 50 mg/ml, preferably at a concentration in the range from about 1 mg/ml to about 40 mg/ml, more preferably from about 1 mg/ml to about 30 mg/ml and most preferably from about 8 mg/ml to about 12 mg/ml.

The formulations of the present invention contain a micelle-forming surfactant that is a pharmaceutically acceptable amphiphilic excipient having hydrophobic and hydrophilic units or groups which are capable of solubilizing a non-water soluble drug in water. HLB values of 10-18 are generally most favorable for solubilization of the echinocandin compounds. The surfactant is present in the formulation at a weight ratio of echinocandin to surfactant from about 1:1.75 to about 1:25, more preferably in a ratio of about 1:2 to about 1:3. The upper limit of surfactant added to the formulation may be limited by its toxicity in application of the pharmaceutical medicament; therefore, the upper limit may vary depending upon the particular surfactant selected. Suitable surfactants include polysorbates (e.g., polysorbate 80, polysorbate 40, polysorbate 20), polyoxyethylene castor oil derivatives (e.g., Chemophors™ (polyoxyl 35 castor oil, polyoxyl 40 hydrogenated castor oil, and polyoxyl 60 hydrogenated castor oil) available from BASF)), polyoxyethylene stearates (e.g., Solutol™ HS 15 (macrogol-660-hydroxystearate, available from BASF), sorbitan trioleate, bile salts (e.g., cholic acids, deoxycholic acids and salts thereof (e.g., sodium deoxycholate or sodium taurodeoxycholate)), lecithin, and the like. Preferred surfactants include polysorbate 80, polysorbate 40,polysorbate 20, and polyoxyethylene hydroxystearates having reduced histamine effects (e.g., Solutol™ HS 15). Surfactants that do not solubilize well include certain poloxamers which are block copolymers of propylene oxide and ethylene oxide.

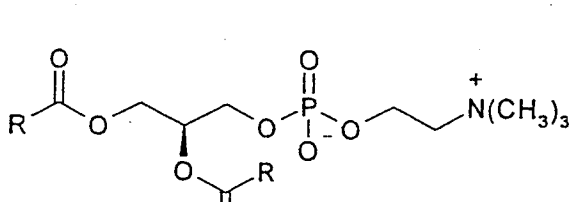
"Polysorbate" refers to materials having the following general structure:



where x+y+w+z is equal to an integer between 5 and 20.

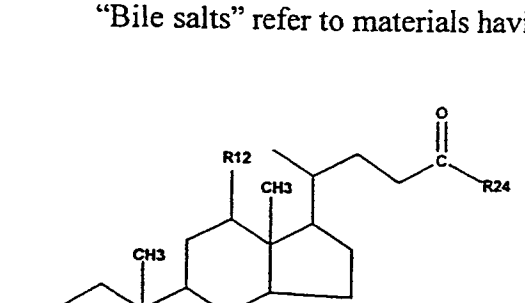
The commercial product Tween™ 20, 40 and 80 (available from ICI Americas Inc., Wilmington, DL) are represented by the above structure when x+y+w+z = 20.

"Lecithin" refers to materials having the following general structure:



R is typically the residue from stearic acid, palmitic acid or oleic acid.

"Bile salts" refer to materials having the following general structure:



where R3, R7, and R12 are -OH, -H or $-SO_3^-$ groups and R24 is -OH or an alkali salt of $-CO_2^-$, $-C(O)NH(CH_2)_nSO_3^-$, or $-C(O)NH(CH_2)_nCO_2^-$ and n is equal to an integer between 1 and 4.

A typical solution formulation includes an echinocandin compound and a micelle-forming surfactant. Applicants have observed that the incorporation of the micelle-forming surfactant not only optimizes the solubilization of the echinocandin compound but also enhances the stability of the solution. The formulation may optionally include one or more of a buffer, a stabilizing agent, and/or a tonicity agent. If the formulation is in the form of a solution then a solvent is also present. Solvents are generally selected based on solvents recognized as safe (GRAS) to be administered parenterally to a mammal. In general, safe solvents are non-toxic aqueous solvents such as, water and other non-toxic solvents that are soluble or miscible in water. Suitable solvents include water, ethanol, propylene glycol,

polyethylene glycols (e.g., PEG400, PEG300), etc. and mixtures thereof. A preferred solvent is water.

A typical freeze-dried formulation includes an echinocandin compound, a pharmaceutically acceptable micelle-forming surfactant, a bulking agent and/or a stabilizing agent. The formulation may optionally include one or more buffering agents. Applicants have observed that the addition of the micelle-forming surfactant not only optimizes the reconstitution of the freeze-dried formulation in a non-toxic aqueous solvent but also provides enhanced stability to the freeze-dried materials.

Both solution and freeze-dried formulations may optionally contain a stabilizing agent. A stabilizing agent is generally present at a concentration in the range from about 0.5% to about 40% (wgt./vol.), more preferably at a concentration in the range from about 1% to about 6%. The term "stabilizing agent" refers to a pharmaceutically acceptable excipient that enhances the chemical or physical stability of the active ingredient in the formulation. Suitable stabilizing agents include polyols (e.g., polyethylene and propylene glycols, and carbohydrates such as sucrose, trehalose, fructose, lactose and mannitol), amino acids and surfactants such as polysorbates and bile salts. Preferred stabilizing agents for freeze dried formulation include mannitol, sucrose, trehalose, fructose, lactose and combinations thereof. In solution most preferred stabilizing agents are the bile salts, polyethylene glycols and propylene glycol.

Both solution and freeze-dried formulations may also optionally contain a buffer. The buffer is present at a concentration in the range from about 0.03% to about 5.0% (wgt./vol.), more preferably at a concentration in the range from about 0.1% to about 1.0%. The term "buffer" refers to a pharmaceutically acceptable excipient that maintains the pH of the solution within a particular range specific to the buffering system. A suitable pH range is from pH 3.0-7.0. Preferably the pH range is from 4.0-5.5, more preferably 4.0-5.0. Suitable buffers include acetates, citrates, phosphates, tartrates, lactates, succinates, amino acids and the like. Preferred buffers for the solution formulation include acetate, citrate, tartrates, phosphate salts and combinations thereof. For the freeze dried formulation, the preferred buffer is tartaric acid.

Solution formulations may optionally contain one or more tonicity agents. The tonicity agent is generally present at a concentration in the range from about 1 to about 100

mg/ml, more preferably in the range from about 9 to about 50 mg/ml. The term "tonicity agent" refers to a pharmaceutically acceptable excipient that makes the solution compatible with blood. Tonicity agents are desirable in injectable formulations. Suitable tonicity agents include glycerin, lactose, mannitol, dextrose, sodium chloride, sodium sulfate, sorbitol and the like. Preferred tonicity agents include mannitol, sorbitol, lactose and sodium chloride and combinations thereof.

When freeze-dried, the formulations may optionally contain a bulking agent. The bulking agent is present in a formulation at a concentration in the range from about 2% to about 10% (wgt./vol.), more preferably at a concentration in the range from about 3% to about 6%. The term "bulking agent" refers to a pharmaceutically acceptable excipient that adds bulk to a formulation which results in a well-formed cake upon freeze drying. Suitable bulking agents include mannitol, glycine, lactose, sucrose, trehalose, dextran, hydroxyethyl starch, ficoll and gelatin. Preferred bulking agents include mannitol, sucrose, trehalose, lactose and combinations thereof.

The formulations may be prepared using conventional dissolution and mixing procedures. For example, the bulk drug substance (e.g., echinocandin compound or echinocandin/carbohydrate complex) is dissolved in a non-toxic aqueous solvent in the presence of a pharmaceutically acceptable micelle-forming surfactant and optionally one or more bulking agents, buffers, stabilizing agents and/or tonicity agents. The resulting solution is then sterile filtered and preferably freeze-dried to provide the desired formulation. Prior to freeze drying, the surfactant is generally present in an amount greater than 1% weight per volume of solution.

Alternatively, the pharmaceutical solution may be prepared by forming an aqueous buffer solution buffered to a pH from about 3.0 to 7.0 (preferably from 4.0 to 5.5, more preferably from about 4.0 to 5.0). The buffer used may be any of the buffers described earlier. The micelle-forming surfactant is then added to the buffered solution and the solution cooled to about 5° to 15°C (preferably about 7° to 10°C). A slurry of the echinocandin compound or echinocandin/carbohydrate complex in a non-toxic aqueous solvent (which may or may not be the same as the solvent used in the buffer solution) is added to the cooled solution containing the surfactant. One or more bulking agents, stabilizing agents and/or tonicity agents may be added to the solution prior to freeze-drying.

The resultant solution is then diluted with addition solvent, filtered and freeze-dried to provide the desired formulation.

A suitable method for freeze-drying is described in Nail et al. Freeze Drying Principles and Practice, in Pharmaceutical Dosage Forms, 2nd Ed., Marcel Dekker, Inc. NY, pp. 163-233 (1993). The formulation is preferably freeze-dried in a vial which can then be stored until needed. A non-toxic, aqueous solvent is added to the vial to dissolve the freeze-dried material thus forming a solution that can be used in a parenteral therapeutic application. Those skilled in the art will appreciate that the aqueous solvent includes other common solutions used in such applications (e.g., saline solutions, dextrose solutions, etc.).

In general, freeze-dried formulations contain a bulking agent and non freeze-dried formulations contain one or more tonicity agents. In application, the formulations are typically diluted or reconstituted (if freeze-dried) and further diluted if necessary, prior to administration. An example of reconstitution instructions for the freeze-dried product are to add ten ml of water for injection (WFI) to the vial and gently agitate to dissolve. Typical reconstitution times are less than one minute. The resulting solution is then further diluted in an infusion solution such as dextrose 5% in water (D5W), prior to administration.

The active ingredient is typically formulated into pharmaceutical dosage forms to provide an easily controllable dosage of the drug and to give the patient an elegant and easily handleable product. Formulations may comprise from 0.1% to 60% by weight of active ingredient, more generally from about 10% to about 30% by weight.

As used herein, the term "unit dose" or "unit dosage" refers to physically discrete units that contain a predetermined quantity of active ingredient calculated to produce a desired therapeutic effect. When a unit dose is administered parenterally, it is typically provided in the form of measured units in ampoules (or vials).

The dosage to be administered may vary depending upon the physical characteristics of the patient, the severity of the patient's symptoms, and the means used to administer the drug. The specific dose for a given patient is usually set by the judgment of the attending physician.

Suitable carriers, diluents and excipients are known in the art and include materials such as carbohydrates, waxes, water soluble and/or swellable polymers, hydrophilic or hydrophobic materials, gelatin, oils, solvents, water, and the like. The particular carrier,

diluent or excipient used will depend upon the means and purpose for which the active ingredient is being applied. The formulations may also include wetting agents, lubricating agents, emulsifiers, suspending agents, preservatives, sweeteners, perfuming agents, flavoring agents and combinations thereof.

A pharmaceutical composition may be administered using a variety of methods. Suitable methods include injection. The particular treatment method used will depend upon the type of infection being addressed.

Echinocandin and semi-synthetic echinocandin compounds have been shown to exhibit antifungal and antiparasitic activity such as growth inhibition of various infectious fungi including Candida spp. (i.e., *C. Albicans, C. Parapsilosis, C. Krusei, C. Glabrata, C. Tropicalis,* or *C. Lusitaniaw*); Torulopus spp. (i.e., *T. Glabrata*); Aspergillus spp. (i.e., *A. Fumigatus*); Histoplasma spp. (i.e., *H. Capsulatum*); Cryptococcus spp. (i.e., *C. Neoformans*); Blastomyces spp. (i.e., *B. Dermatitidis*); Fusarium spp.; Trichophyton spp., *Pseudallescheria boydii, Coccidioides immits, Sporothrix schenckii,* etc.

Compounds of this type also inhibit the growth of certain organisms primarily responsible for opportunistic infections in immunosuppressed individuals, such as growth inhibition of *Pneumocystis carinii* (the causative organism of pneumocystis pneumonia (PCP) in AIDS and other immunocompromised patients. Other protozoans that are inhibited by echinocandin-type compounds include Plasmodium spp., Leishmania spp., Trypanosoma spp., Cryptosporidium spp., Isospora spp., Cyclospora spp., Trichomnas spp., Microsporidiosis spp., etc.

Consequently, the formulations of the present invention are useful in combating either systemic fungal infections or fungal skin infections. Accordingly, the processes and formulations of the present invention may be used in the manufacture of a medicament for the therapeutic applications described herein. For example, fungal activity (preferably, *Candida albicans* or *Aspergillus fumigatis* activity) or parasitic activity may be inhibited by contacting a pharmaceutical formulation prepared by the present invention with a fungus or parasite, respectively. The term "contacting" includes a union or junction, or apparent touching or mutual tangency of a compound of the invention with a parasite or fungus. The term does not imply any further limitations to the process, such as by mechanism of inhibition. The methods are defined to encompass the inhibition of parasitic and fungal

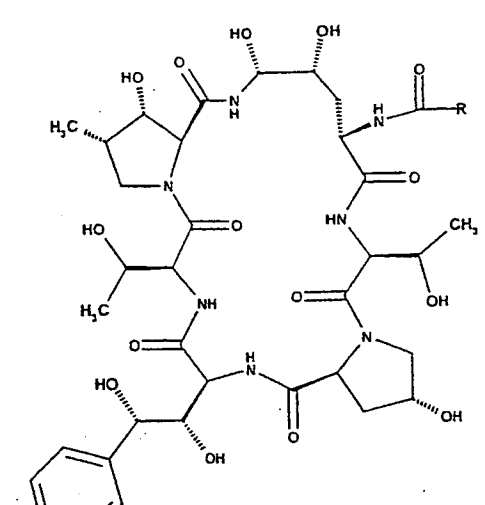
activity by the action of the compounds and their inherent antiparasitic and antifungal properties.

A method for treating a fungal infection which comprises administering an effective amount of a pharmaceutical formulation of the present invention to a host in need of such treatment is also provided. A preferred method includes treating a *Candida albicans* or *Aspergillus fumigatis* infection. The term "effective amount" refers to an amount of active compound which is capable of inhibiting fungal activity. The dose administered will vary depending on such factors as the nature and severity of the infection, the age and general health of the host and the tolerance of the host to the antifungal agent. The particular dose regimen likewise may vary according to these factors. The medicament may be given in a single daily dose or in multiple doses during the day. The regimen may last from about 2-3 days to about 2-3 weeks or longer. A typical daily dose (administered in single or divided doses) contains a dosage level between about 0.01 mg/kg to 100 mg/kg of body weight of an active compound. Preferred daily doses are generally between about 0.1 mg/kg to 60 mg/kg and more preferably between about 2.5 mg/kg to 40 mg/kg.

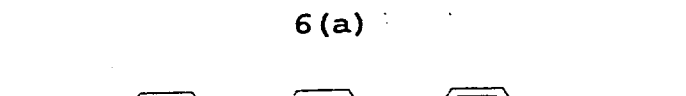
The following examples are provided to illustrate but not limit the invention. All references cited herein are hereby incorporated herein by reference.

EXAMPLES

The echinocandin compound used to exemplify the formulations of the present invention was prepared as described in the following preparations. Specifically, the following sequence describes the preparation of anti-fungal compound 6(a) having the following structure:



6 (a)



It will be understood by those skilled in the art that the following serves as an illustrative example and that other semi-synthetic echinocandin compounds useful as anti-fungal agents may be synthesized using similar procedures or procedures described in references cited earlier in the specification. Materials used in the following preparations are available from Aldrich Chemicals (Milwaukee, Wisconsin) unless designated otherwise.

Compound Preparations

Preparation of 4-Bromo-4'-pentyloxybiphenyl 1(a)

Anhydrous K_2CO_3 (416g, 3mol) was added to a mixture of 4-bromo-4'-hydroxybiphenyl (300g, 1.2mol), 1-iodopentane (234ml, 1.79mol) and 2-butanone (600ml). The reaction mixture was refluxed for 44h until TLC (85:15 hexanes/EtOAc) showed complete consumption of the bromo alcohol. The mixture was cooled to about 30°C, diluted with CH_2Cl_2 (600ml) and then filtered. The filtrate was washed twice with H_2O and twice with a saturated aqueous NaCl solution, dried over anhydrous Na_2SO_4, filtered and then dried at reduced pressure to provide a solid. This solid was isolated by filtration,

washed repeatedly with a total of 2L of ice-cold heptane to remove all traces of iodopentane and then dried overnight under high vacuum. Yield: 340g (88%) of a white powder.

Alternative Preparation of 4-bromo-4'-pentyloxybiphenyl 1(a):

4-Bromo-4'-hydroxybiphenyl (12.5g, 50.2mmol) was added to a solution of NaOH (2.28g, 97% pure, 55.2mmol) in deionized H_2O (150ml), followed by the addition of 1-iodopentane (11.9g, 60.2mmol) and tetrabutylammonium bromide (0.82g, 2.51mmol). The mixture was stirred at 90°C for 3.75h until the solids went into solution. Then, as the reaction proceeded, the desired product began to precipitate. The mixture was slowly cooled and then filtered to provide a solid which was washed with deionized water until the pH of the filtrate was neutral and then dried for 16h in a vacuum oven at 30°C.

Yield: 15.41g (96%) of 5a. R_f 0.5 (97:3 hexanes/EtOAc). ^{1}H NMR: δ 0.93 (t, 3H, J=6.9Hz); 1.41 (m, 4H); 1.79 (m, 2H); 3.97 (t, 2H, J= 6.6Hz); 6.98 (m, 2H); 7.23 (m,6H). ^{13}C NMR: δ 14.03; 22.43; 28.22; 28.98; 68.12; 114.91; 120.71; 127.93; 128.27; 131.77; 132.24; 139.82; 159.03. MS(FAB$^+$): m/z 320. IR($CHCl_3$): 2960, 2936, 2874, 1608, 1518, 1485, 1475 cm^{-1}. Analysis for $C_{17}H_{19}BrO$: Calcd: C, 63.96; H. 6.00; Br, 25.0; Found: C, 64.10; H. 5.97; Br, 25.28.

Preparation of 4-Boronic acid-4'-pentyloxybiphenyl 2(a):

To a cold (-20°C) mixture of Compound 1(a) (100g, 0.31mol) in *t*-butylmethylether (MTBE) (1L), was slowly added n-butyl lithium (150ml of a 2.5M hexanes solution, 0.37mol) dropwise under N_2, while maintaining the internal temperature between -19° and -18°C. The resultant mixture was stirred for 3.5h between -17° and -16°C which resulted in a light yellow-green solution. This solution was cooled to -78°C and diluted with 100ml of anhydrous THF which resulted in a white precipitate. Then, a cold (-78°C) solution of triisopropylborate (145ml, 0.62mol) in MTBE (200ml), under nitrogen was added dropwise over 1.5h while maintaining the reaction temperature between -78° and -74°C. The resultant reaction mixture was stirred for 1.5h at -78°C, then allowed to warm to -50°C over 1h at which time the cooling bath was removed and the mixture was stirred overnight (16-21h) which resulted in a white precipitate. The mixture was shaken vigorously with 2M HCl (1000ml) for 5 minutes and then the resulting layers were separated and the organic layer was dried at reduced pressure to provide a residue. This residue was diluted with

MTBE (100ml), followed by heptane (800ml) to provide a white powder which isolated by suction filtration and washed 3 times with heptane (300ml).

Yield: 88g (98%). R_f 0.45 (95:5 CH_2Cl_2/MeOH). ^1H NMR: δ 0.92 (m, 3H); 1.41 (m, 4H); 1.80 (m, 2H); 4.00 (m, 2H); 6.99 (m, 2H); 7.45-7.63 (m, 3H); 7.67 (m, 2H); 8.24 (d, 1H, J=8.3Hz). ^{13}C NMR: 14.01; 22.26; 28.03; 28.77; 39.61; 39.89; 40.17; 40.45; 67.82; 114.77; 125.32; 127.83; 132.93; 134.84; 141.88; 158.71. MS(FD$^-$): m/z 284. IR($CHCl_3$): 2959, 2952, 2874, 1606, 1526, 1500 cm^{-1}.

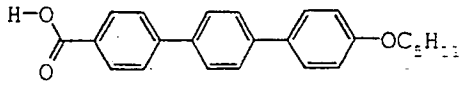
Preparation of Compound 3(a):



3(a)

A solution of toluene (174ml) and propanol (20ml) was degassed 3 times by applying vacuum to the solution for 20-30 seconds followed by purging with N_2. A 2M solution of Na_2CO_3 was also degassed. The toluene/propanol solution (97ml) was added to a mixture of methyl 4-iodobenzoate (14.12g, 53.9mmol) and Compound 2(a) (15.0g, 52.8mmol), followed by a degassed 2M aqueous Na_2CO_3 solution (29ml, 58.0mmol). The resultant mixture was degassed 2 times for 20-30 seconds each under a positive pressure of N_2, followed by the addition of palladium (II) acetate (0.24g, 1.1mmol) and triphenylphosphine (0.84g, 3.2mmol) and then degassed 2 more times. The reaction mixture was then refluxed under N_2 for 5h resulting in a light-yellow mixture. This mixture was cooled to 23°C resulting in the formation of a precipitate which was collected by filtration, washed successively with toluene (123ml), 2:1 MTBE/EtOAc (143ml), deionized water (123ml) and 2:1 MTBE/EtOAc (42ml) and then dried for 16h in a vacuum oven at 35°C. Yield: 18.7g (94%). R_f 0.48 (benzene). ^1H NMR: δ 0.93 (t, 3H, J=6.80Hz); 1.42 (m, 4H); 1.81 (m, 2H); 3.95 (s, 3H); 4.00 (t, 2H, J= 6.48 Hz); 6.97 (d, 2H, J=8.52Hz); 7.55 (d, 2H, J= 8.52Hz); 7.66 (m, 6H), 8.10 (d, 2H, J=8.20Hz). MS(FD$^+$): m/z 374. IR(KBr): 2938, 1723 cm^{-1}. Analysis for $C_{25}H_{26}O_3$: Calcd: C, 80.18; H. 7.00; Found: C, 79.91; H. 6.94.

Preparation of Compound 4(a):



4 (a)

A mixture of Compound 3(a) (80g, 0.21mol), 5M KOH (160ml) and cetyltrimethylammonium bromide (4.8g, 0.013mol) in xylene (800ml) was refluxed for 3h and then cooled to 10°C and filtered to provide a white solid. This solid was washed 3 times with H_2O (500ml each) to remove the catalyst and most of the base. The resultant material was treated with DME (500 ml). The pH of the solution was adjusted to pH by the addition of 6M HCl (100ml). The resultant mixture was refluxed for 30 minutes while periodically checking the pH to assure that it remained acidic, then cooled and filtered. The resulting solid was washed successively with MTBE (400ml) and water (4x400ml) until the washings were neutral to litmus. Yield: 76 g (98% yield). ^{1}H NMR δ 0.89 (t, 3H, J= 6.82Hz), 1.38 (m, 4H), 1.73 (m, 2H), 3.96 (t, 2H, J= 6.3Hz), 6.95 (d, 2H, J=8.56Hz), 7.57 (d, 2H, J=8.54Hz), 7.64-7.74 (m, 6H), 8.00 (d, 2H, J=8.21Hz), 8.09 (s, 1H). MS(FD$^+$) m/z 360. IR(KBr): 2958, 2937, 2872, 1688 cm^{-1}. Analysis for $C_{24}H_{24}O_3$: Calcd: C, 79.97; H. 6.71; Found: C, 80.50; H. 6.77.

Preparation of HOBT ester of Compound 4(a):

A. Formation of HOBT mesylate

To a cold (0°C) mixture of hydroxybenzotriazole hydrate (200g, 1.48mol) in anhydrous CH_2Cl_2 (1.5L), was slowly added anhydrous Et_3N (268ml, 1.92mol) while maintaining a temperature of 0-10°C, followed by the addition of methanesulfonyl chloride (126ml, 1.63mol) while maintaining a temperature of 0-5°C. The resultant mixture was stirred for 3h at 0°C and washed successively with cold water (2 x 1.2L) and brine (1.2L). The combined organic extracts were concentrated at reduced pressure to provide a solid. This solid was recrystallized from CH_2Cl_2 (100ml) and heptane (1L). The crystals were collected by suction filtration and washed repeatedly with a total of 1.L of heptane and then dried overnight under high vacuum (0.5 mm Hg). Yield: 245g (78%) R_f 0.55 (1:1 hexanes/CH_2Cl_2). ^{1}H NMR: δ 3.58 (s, 3H), 7.46 (t, 1H, J= 7.60Hz), 7.60 (d, 1H, J= 8.28 Hz), 7.65 (d, 1H, J= 8.56Hz), 7.68 (d,1H, J= 8.20 Hz), 8.05 (d, 1H, J=8.41Hz).

B. Formation of HOBT ester

A mixture of Compound 4(a) (50g, 0.14mol) and the material described above in part A (36g, 0.17mol) in DMF (650ml) was treated dropwise with Et_3N (25ml, 0.18mol), under N_2. The resultant mixture was stirred for 4h at room temperature until all the acid was consumed, as determined by TLC (95:5 CH_2Cl_2/MeOH). When all the acid was consumed, an aliquot of the reaction mixture (~3 pipes drops) gave a clear homogeneous solution when diluted with 3ml of 1:1 CH_2Cl_2/THF. The reaction mixture was then diluted with toluene (500ml), washed with water (500ml). The organic layer (containing solid product) was diluted with water (500ml) and filtered using MTBE for transferring. The solid was rinsed with MTBE (2 x 400ml) and dried under vacuum to provide green-white flakes of material. NOTE: This material could be dissolved in THF and filtered to remove any remaining metal contamination. Yield: 61g (92%). R_f 0.68 (1:1 CH_2Cl_2/hexanes). ^1H NMR: δ 0.93 (t, 3H, J=7.0Hz), 1.42 (m, 4H), 1.81 (m, 2H), 4.00 (t, 2H, J=6.53Hz), 6.99 (d, 2H, J=8.6Hz), 7.42-7.59 (m, 5H), 7.71 (dd, 4H, J=13.91Hz, 8.40Hz), 7.86 (d, 2H, J=8.30Hz), 8.11 (d, 1H, J= 8.31Hz), 8.35 (d, 2H, J=8.33Hz). ^{13}C NMR: δ 14.03, 22.44, 28.18, 28.94, 40.10, 40.37, 68.11, 108.45, 110.11, 114.95, 118.71, 120.48, 123.04, 124.94, 124.99, 127.00, 127.23, 127.51, 127.73, 128.06, 128.82, 128.86, 131.35, 132.30, 137.15, 141.43, 143.54, 147.85, 159.15, 162.73. MS(FD^+): m/z 477. IR($CHCl_3$): 2960, 2936, 2874, 1783, 1606 cm^{-1}. Analysis for $C_{30}H_{27}N_3O_3$: Calcd: C, 75.45; H, 5.70; N, 8.80; Found: C, 75.69; H, 5.58; N, 8.92.

Preparation of Anti-fungal Compound 6(a):

Deionized water was used throughout the procedure. A mixture of Compound 5(a) (11g, 23mmol) and the nucleus of Compound 6(a) (where R is hydrogen - 92% pure by HPLC, 19.25 g, 22.2mmol) in anhydrous DMF (275ml) was stirred, under N_2 for 4h (until HPLC showed complete consumption of the cyclic peptide starting material). The mixture was filtered through a bed of celite and the filtrate was concentrated under reduced pressure at 35°C to provide a paste that could be stirred. This paste was poured into MTBE (500ml) which resulted in the precipitation of a fine powder which was collected by vacuum filtration and dried to provide 27g of crude material. This material was crushed to a powder with a mortar and pestle, slurried for 5 minutes in toluene (200ml), suction filtered

(slow filtered), rinsed with MTBE (100ml) and then dried *in vacuo* to provide a yellow solid. Yield: 23 g (95% pure by HPLC, retention time = 7.79 min).

Alternatively, the conversion may be carried out using an excess of the cyclic nucleus (1.1 equiv.). When the reaction is substantially complete, as indicated by HPLC, the crude material (10g of a powder) is added portion-wise to a vigorously stirred mixture of 9:1 acetone/water (60ml). Celite (2.5 g, pre-washed with a 9:1 acetone/water mixture) is added to the resultant suspension. After stirring for 2 minutes, the mixture is filtered through a bed of celite (prewashed with 9:1 acetone/water) and the cake is rinsed twice with 9:1 acetone/water (10ml). The filtrate is poured into a beaker of deionized water (200ml) while gently swirling the mixture which resulted in the formation of a precipitate. This precipitate is collected by suction filtration, rinsed with H_2O (4 x 25ml), and then dried *in vacuo* at room temperature. Yield: 6.81g (97% pure by HPLC).

The product was further purified using preparatory HPLC chromatography. R_f 0.29 (80:20 $CHCl_3$/MeOH). MS(FAB$^+$): m/z for $C_{58}H_{74}N_7O_7$, Calcd: 1140.5141; Found: 1140.5103. IR(KBr): 3365, 2934, 1632, 1518 cm^{-1}.

Pharmaceutical Formulations

The following Examples illustrate the formulations of the invention and methods for their preparation. The Examples are not intended to limit the scope of the invention in any respect and should not be so construed.

The following formulations were prepared by Method A, Method B or Method C. The quantity of Compound 6(a) was determined by calculating the theoretical potency needed for the experiments and dividing that value by the "as-is" HPLC potency of the compound.

Method A After adjusting the pH of 50 ml of a 0.1M citrate buffer stock solution to pH 4, 2.5g of polysorbate 80 was added and the resultant mixture was mixed until dissolved, followed by the addition of 1g of the compound of Preparation 6(a) (potency). The resultant mixture was mixed until dissolved, followed by the addition of 3g of mannitol (bulking agent) and 2g of trehalose (stabilizing agent). The resultant mixture was again mixed until dissolved. The resultant solution was diluted with water to 100 ml in a volumetric flask. A 3 ml sample of this solution was transferred to a vial and then freeze-dried in a lyophilizer.

Method B The pH of a solution of 0.3005g of acetic acid in 50 ml of water was adjusted to pH 4.0 using a 1N sodium hydroxide solution. While mixing, 2.5g of polysorbate 80 and 5.0g of mannitol (bulking agent) were added and the resultant mixture was mixed until dissolved, followed by the addition of 1g of Compound 6(a) (potency). The resultant mixture was again mixed until dissolved. The resultant solution was diluted with water to 100 ml in a volumetric flask, filtered and then filled into vials. The vials may be freeze-dried in a lyophilizer or stored at 5°C.

Method C The pH of a solution of 0.113 g of tartaric in 50 ml of water was adjusted to pH 4.3 using a 10% sodium hydroxide solution. While mixing, 2.5 g of polysorbate 80 was added and mixed until dissolved. The temperature of the solution was reduced to 5-15°C, followed by the addition of 1 g of Compound 6(a) (potency) as a slurry in water. The resultant mixture was again mixed until dissolved followed by the addition of 1.0 g of fructose (stabilizing agent) and 5.0g of mannitol (bulking agent). The resultant mixture was again mixed until dissolved. The resultant solution was diluted with water to 100 ml in a volumetric flask, filtered and then filled into vials. The vials may be freeze-dried in a lyophilizer or stored at 5°C.

The following Formulation Examples 1-27 were prepared substantially in accordance with Methods A and B, described above. The symbol "-" means that the designated ingredient was omitted from the formulation. In Table 1 and subsequent tables, all weights are in (grams); concentrations ([]) are in mg/ml; CA stands for citric acid; Y stands for yes; N stands for no; Man stands for mannitol; Tre stands for trehalose; Col. stands for Collapse Cake; C stands for citrate; BA stands for bulking agent; B stands for buffer; PEG is polyethylene glycol; PPG is polypropylene glycol; SA stands for stabilizing agent; Ace is acetate; Poly 80 is polysorbate 80; Com is compound; Suc is sucrose; His is histidine; Sur is surfactant; Lac is lactose; Fru is fructose; TA is tartaric acid; and AA is acetic acid.

Table 1

Ex No	Comp 6(a)	Poly 80	BA	B	SA	Col.
1	1	2.5	Man(3)	CA(0.960)	Tre(2)	Y
2	1	2.5	Man(3)	CA(0.960)	Suc(2)	Y
3	1	2.5	-	CA(0.960)	Suc(5)	Partial
4	1	2.5	-	CA(0.960)	Tre(5)	Partial

5	1	2.5	-	-	Tre(5)	Partial
6	1	2.5	-	-	Suc(5)	Partial
7	1	2.5	-	-	Lac(5)	Partial
8	1	2.5	Man(5)	-	-	N
9	1	2.5	Man(5)	-	Suc(1)	N
10	1	2.5	Man(5)	-	Lac(1)	N
11	1	2.5	Man(5)	CA(0.096)	Lac(1)	N
12	1	2.5	Man(5)	TA(0.113)	Lac(1)	N
13	1	2.5	Man(5)	TA(0.113)	Fru(1)	N
14	1	2.5	Man(5)	-	PEG 8000(1)	N
15	1	2.5	Man(5)	-	PEG 6000(2)	N
16	1	2.5	Man(5)	-	His(0.5) Suc(0.5)	N
17	1	2.5	Man(5)	CA(0.960)	Suc(0.5)	N
18	1	2.5	Man(5)	CA(0.960)	Suc(1)	N
19	1	2.5	Man(5)	AA(0.3)	-	N
20	0.1	2.5	Man(5)	AA(0.3)	-	N
21	0.1	2.5	Man(5)	AA(0.6)	-	N
22	0.1	2.5	Man(5)	AA(0.06)	-	N
23	0.1	2.5	Man(5)	CA(1.92)	-	N
24	0.1	2.5	Man(5)	CA(0.19)	-	N
25	0.1	2.5	Man(5)	CA(0.960)	-	N
26	0.5	1.25	Man(5)	-	Lac(1)	N
27	0.8	2.0	Man(5)	-	Lac(1)	N

* determined by visual observation

Solubility Studies

Solubility studies were performed at room temperature by transferring 5 ml of test solution (surfactant in water optionally containing a bulking agent, a buffer or a stabilizing agent) and 50 mg of Com 6(a) into a glass test tube. The test tubes were agitated overnight and examined for excess solid. Test tubes containing excess solid were removed for analysis. An additional 50mg Compound 6(a) was added to test tubes which did not contain excess solids and the resultant mixture was agitated again. This process was repeated until excess solid was observed in the test tube. All samples were allowed to stand 1h, supernatant was removed, filtered and analyzed by reverse phase HPLC to determine the potency of Com 6(a) per Ml of solution.

Table 2

Sur(s)	[Sur]	Comp6(a) Potency
None	-	<0.1
Poly 80	5	1
Poly 80	10	4.4
Poly 80	15	7.2
Poly 80	25	13.2
Poly 20/ Poly 80	5 5	4.8
Poly 20/ Poly 80	5 10	8.0

ethoxylated castor oil (cremophor™ EL)	25	11.0
Poly 20/ pluronic™ F68	5 10	1.13
Poly 80/sodium taurodeoxycholate	20 20	43.12

The stability of solution and freeze-dried formulations was evaluated by monitoring a sample of the formulation for the percent increase in related substances by reverse phase HPLC at initial, 2 weeks and 4 weeks of storage at 40°C.

Table 3

6(a) Potency (mg/ml)	Sur	B	Ton.Agent	Co-solvent (wgt%/vol)	Percent Related Substances		
					0 wks	2 wks	4 wks
1	Poly 80(25)	Cit(1.9)	Man(50)	-	3.42	8.91	18.04
1	Poly 80(25)	Cit(9.6)	Man(50)	-	3.36	9.25	17.29
1	Poly 80(25)	Cit(19.2)	Man(50)	-	3.47	9.70	18.02
1	Poly 80(25)	Ace(0.6)	Man(50)	-	3.15	11.37	19.44
1	Poly 80(25)	Ace(3)	Man(50)	-	3.33	12.20	18.29
1	Poly 80(25)	Ace(6)	Man(50)	-	3.27	11.07	19.59
10	Poly 80(25)	Ace(3)	-	-	2.51	9.46	14.39
10	Poly 80(20) Poly 20(5)	Ace(6)	-	-	2.65	11.06	15.44
10	Poly 80(15) Poly 20(10)	Ace(6)	-	-	2.70	10.55	15.34
10	Poly 80(5) Poly 20(20)	Ace(6)	-	-	2.67	9.77	15.57
10	Poly 80(25)	Ace(6)	-	20% PEG400	2.83	9.69	14.35
10	Poly 80(25)	Ace(6)	-	40% PEG400	2.57	10.38	13.36
10	Poly 80(25)	Ace(6)	-	20% PPG	2.71	10.58	13.40
10	Poly 80(25)	Ace(6)	-	40% PPG	2.72	9.84	12.65
10	Poly 80(25)	-	-	-	3.62	18.71	27.91
10	Poly 80(25)	-	Man(50)	-	3.68	16.65	23.68
10	Poly 80(25)	-	-	40% PEG 400	3.73	15.26	23.99
10	Poly 80(25)	-	-	1% PEG 3350	3.68	21.56	-

Table 4

6(a) Potency	Sur	B	SA	BA	% Related Substances		
					0 wks	2 wks	4 wks
1	Poly 80(25)	Cit(1.9)	-	Man(50)	3.22	10.24	12.16
1	Poly 80(25)	Cit(9.6)	-	Man(50)	3.36	11.74	18.63
1	Poly 80(25)	Cit(19.2)	-	Man(50)	3.37	17.29	22.61
10	Poly 80(25)	Cit(9.6)	Suc(50)	-	3.60	5.4	6.01
10	Poly 80(25)	Cit(9.6)	Tre(50)	-	3.73	4.83	6.19
10	Poly 80(25)	-	-	Man(50)	3.98	4.85	5.45
10	Poly 80(25)	Cit(9.6)	Suc(5)	Man(50)	3.70	5.60	6.01
10	Poly 80(25)	Cit(9.6)	Suc(10)	Man(50)	3.77	5.41	5.88
10	Poly 80(25)	Tar(1.13)	Fru(10)	Man(50)	2.49	-	2.42

10	Poly 80(25)	-	Suc(10)	Man(50)	4.26	4.82	4.49
10	Poly 80(25)	-	Lac(10)	Man(50)	4.56	4.51	4.85
10	Poly 80(25)	-	Lac(10)	Man(50)	4.06	5.47	5.17
5	Poly 80(12.5)	-	Lac(10)	Man(50)	6.42	6.98	6.85
8	Poly 80 (20)	-	Lac(10)	Man(50)	5.99	6.16	5.74
10	Poly 80(25)	-	PEG8000(10)	Man(50)	3.97	4.23	6.73
10	Poly 80(25)	-	PEG6000(10)	Man(50)	4.13	4.47	8.11

We claim:

1. A parenteral pharmaceutical formulation comprising

(i) an echinocandin compound, or a pharmaceutically acceptable salt thereof;

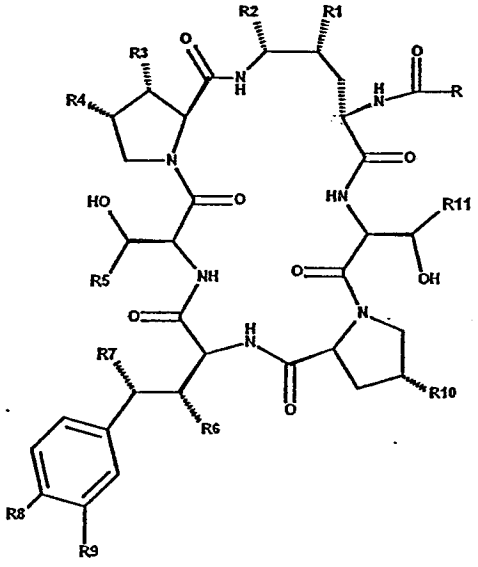
(ii) a pharmaceutically acceptable micelle-forming surfactant;

(iii) a non-toxic, aqueous solvent; and

(iv) a bulking agent.

wherein said surfactant is present in said formulation at a weight ratio of echinocandin compound to micelle-forming surfactant from about 1:1.75 to about 1:25 and said echinocandin compound is present in an amount greater than or equal to 1 mg/ml.

2. The formulation of Claim 1 wherein said echinocandin compound is represented by the following structure:



wherein:

R is an alkyl group, an alkenyl group, an alkynyl group, an aryl group, heteroaryl group, or combinations thereof;

R_1, R_2, R_3, R_6, R_7, and R_{10} are independently hydroxy or hydrogen;

R_4 is hydrogen, methyl or $-CH_2C(O)NH_2$;

R_5 and R_{11} are independently methyl or hydrogen;

R_8 is $-OH$, $-OPO_3H_2$, $-OPO_3HCH_3$, $-OPO_2HCH_3$, or $-OSO_3H$;

R_9 is -H, -OH, or $-OSO_3H$; and

pharmaceutically acceptable salts thereof.

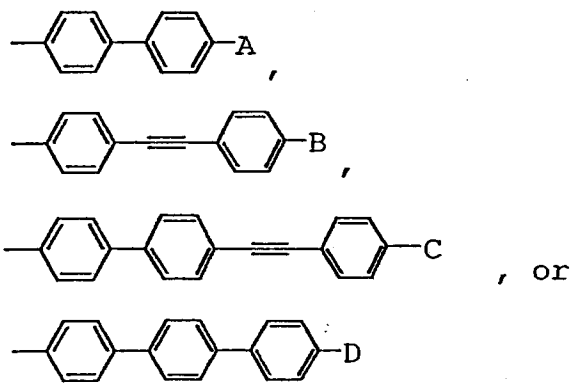
3. The formulation of Claim 2 wherein

R_4, R_5 and R_{11} are each methyl;

R_2 and R_7 are independently hydrogen or hydroxy; R_1, R_3, R_6 and R_{10} are each hydroxy;

R_8 is -OH, $-OPO_3HCH_3$, or $-OPO_2HCH_3$;

R is linoleoyl, palmitoyl, stearoyl, myristoyl, 12-methylmyristoyl, 10,12-dimethylmyristoyl, or a group having the general structure:



, or

where A, B, C and D are independently hydrogen, C_1-C_{12} alkyl, C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, C_1-C_{12} alkylthio, halo, or $-O-(CH_2)_m-[O-(CH_2)_n]_p-O-(C_1-C_{12}$ alkyl) or $-O-(CH_2)_q$-X-E;

m is 2, 3 or 4;

n is 2, 3 or 4; p is 0 or 1; q is 2, 3 or 4;

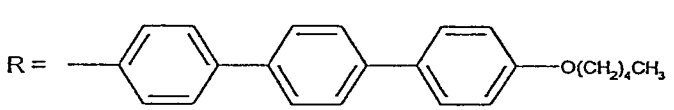
X is pyrrolidino, piperidino or piperazino;

E is hydrogen, C_1-C_{12} alkyl, C_3-C_{12} cycloalkyl, benzyl or C_3-C_{12} cycloalkylmethyl.

4. The formulation of Claim 2 or 3 wherein

R_2 and R_7 are each hydroxy;

R_8 is hydroxy; and

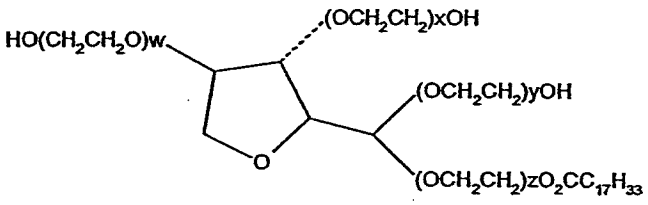


5. The formulation of Claim 1 wherein said micelle-forming surfactant is selected from the group consisting of polysorbates, polyoxyethylene castor oil derivatives, polyoxyethylene stearates, sorbitan trioleate, bile salts, lecithin and combinations thereof.

6. The formulation of Claim 1 wherein said echinocandin compound or a pharmaceutically acceptable salt thereof, is present in an amount from about 1 mg/ml to about 50 mg/ml.

7. The formulation of Claim 1 or 6 wherein said echinocandin compound or a pharmaceutically acceptable salt thereof, is present in an amount from about 1 to about 30 mg/ml.

8. The formulation of Claim 1 wherein said surfactant is represented by the following formula:



wherein x+y+z+w is equal to an integer between 5 and 20.

9. The formulation of Claims 1 or 8 wherein said surfactant is present in an amount greater than 1% weight per volume.

10. The formulation of Claims 1, 7 or 8 wherein said weight ratio of echinocandin to surfactant is from about 1:2 to about 1:3.

11. The formulation of Claim 1 wherein said solvent is selected from the group consisting of water, ethanol, propylene glycol, polyethylene glycols and mixtures thereof.

12. The formulation of Claim 1 further comprising a stabilizing agent.

13. The formulation of Claim 12 wherein said stabilizing agent is present in an amount from about 0.5% to about 10% by weight per volume.

14. The formulation of Claims 12 or 13 wherein said stabilizing agent is present in an amount from about 1% to about 6% by weight per volume.

15. The formulation of Claim 12, 13 or 14 wherein said stabilizing agent is selected from the group consisting of mannitol, histidine, lysine, glycine, sucrose, fructose, trehalose, lactose and mixtures thereof.

16. The formulation of Claim 1 further comprising a buffer.

17. The formulation of Claim 16 wherein said buffer is selected from the group consisting of acetates, citrates, tartrates, lactates, succinates and phosphates and amino acids.

18. The formulation of Claim 1 further comprising a tonicity agent.

19. The formulation of Claim 18 wherein said tonicity agent is selected from the group consisting of glycerin, lactose, mannitol, dextrose, sodium chloride, sodium sulfate and sorbitol.

20. The formulation of Claims 18 or 19 wherein said tonicity agent is present in amount from about 1 to about 100 mg/ml.

21. The formulation of Claims 18, 19 or 20 wherein said tonicity agent is present in amount from about 9 to 50 mg/ml.

22. A freeze-dried formulation comprising

(i) an echinocandin compound, or a pharmaceutically acceptable salt thereof;

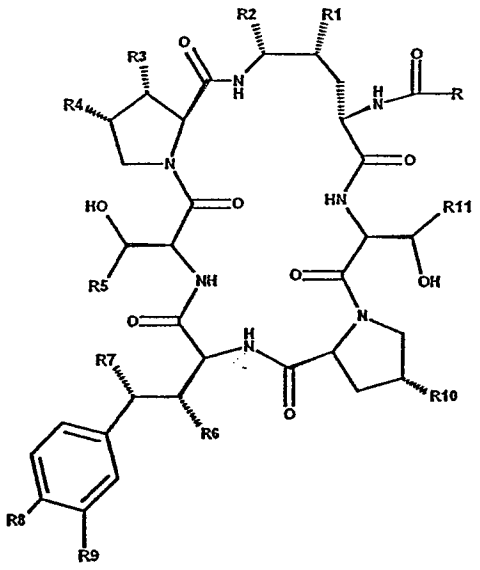
(ii) a pharmaceutically acceptable micelle-forming surfactant; and

(iii) a bulking agent,

wherein said micelle-forming surfactant is present in said freeze-dried formulation in an amount greater than 5% by weight.

23. The formulation of Claim 22 wherein said bulking agent is selected from the group consisting of mannitol, sucrose, trehalose, lactose and mixtures thereof.

24. The formulation of claim 22 wherein said echinocandin compound is represented by the following structure:



wherein:

R is an alkyl group, an alkenyl group, an alkynyl group, an aryl group, heteroaryl group, or combinations thereof;

R_1, R_2, R_3, R_6, R_7, and R_{10} are independently hydroxy or hydrogen;

R_4 is hydrogen, methyl or $-CH_2C(O)NH_2$;

R_5 and R_{11} are independently methyl or hydrogen;

R_8 is -OH, $-OPO_3H_2$, $-OPO_3HCH_3$, $-OPO_2HCH_3$, or $-OSO_3H$;

R_9 is -H, -OH, or $-OSO_3H$; and

pharmaceutically acceptable salts thereof.

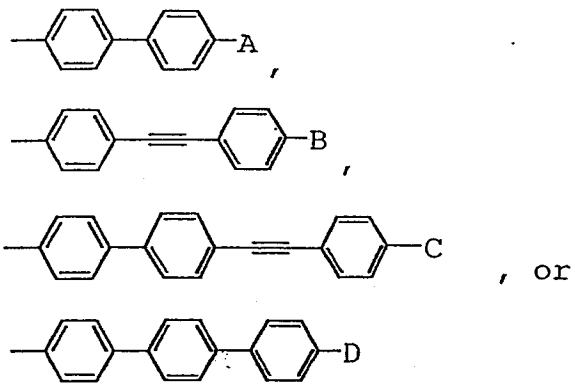
25. The formulation of claim 24 wherein

R_4, R_5 and R_{11} are each methyl;

R_2 and R_7 are independently hydrogen or hydroxy; R_1, R_3, R_6 and R_{10} are each hydroxy;

R_8 is -OH, $-OPO_3HCH_3$, or $-OPO_2HCH_3$;

R is linoleoyl, palmitoyl, stearoyl, myristoyl, 12-methylmyristoyl, 10,12-dimethylmyristoyl, or a group having the general structure:



, or

where A, B, C and D are independently hydrogen, C_1-C_{12} alkyl, C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, C_1-C_{12} alkylthio, halo, or -O-$(CH_2)_m$-[O-$(CH_2)_n$]$_p$-O-(C_1-C_{12} alkyl) or -O-$(CH_2)_q$-X-E;

m is 2, 3 or 4;

n is 2, 3 or 4; p is 0 or 1; q is 2, 3 or 4;

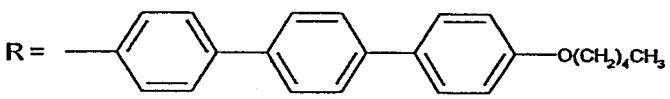
X is pyrrolidino, piperidino or piperazino;

E is hydrogen, C_1-C_{12} alkyl, C_3-C_{12} cycloalkyl, benzyl or C_3-C_{12} cycloalkylmethyl.

26. The formulation of Claims 24 and 25 wherein

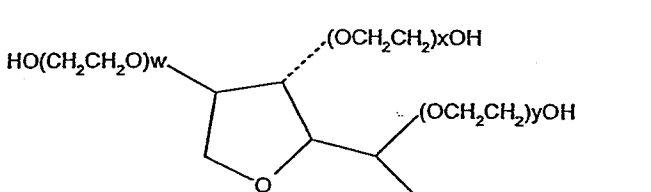
R_2 and R_7 are each hydroxy;

R_8 is hydroxy; and



27. The formulation of Claim 22 wherein said micelle-forming surfactant is selected from the group consisting of polysorbates, polyoxyethylene castor oil derivatives, polyoxyethylene stearates, sorbitan trioleate, bile salts, lecithin and combinations thereof.

28. The formulation of Claim 22 wherein said surfactant is represented by the following formula:



wherein x+y+z+w is equal to an integer between 5 and 20.

29. The formulation of Claim 22 wherein said surfactant is present in said formulation at a weight ratio of echinocandin to surfactant from about 1:1.75 to about 1:25.

30. The formulation of Claims 22 or 29 wherein said weight ratio of echinocandin to surfactant is from about 1:2 to about 1:3.

31. A parenteral formulation comprising the freeze-dried formulation of Claims 22 - 30 and an aqueous solvent.

32. The formulation of Claim 31 further comprising a stabilizing agent.

33. The formulation of Claim 32 wherein said stabilizing agent is selected from the group consisting of mannitol, histidine, lysine, glycine, fructose, sucrose, trehalose, lactose and mixtures thereof.

34. The formulation of Claim 31 wherein said surfactant is present in said formulation at a weight ratio of echinocandin to surfactant from about 1:1.75 to about 1:25.

35. The formulation of Claim 31 further comprising a buffer.

36. The formulation of claim 35 wherein said buffer is selected from the group consisting of acetates, tartrates, citrates, phosphates and amino acids.

37. A process for preparing a parenteral formulation comprising the step of mixing an echinocandin compound or an echinocandin/carbohydrate complex containing said echinocandin compound and a pharmaceutically acceptable micelle-forming surfactant in an aqueous solvent, wherein said micelle-forming surfactant is present in said formulation at a weight ratio of echinocandin compound to surfactant from about 1:1.75 to about 1:25 and said echinocandin compound is present in an amount greater than or equal to 1 mg/ml.

38. The process of Claim 37 wherein said echinocandin compound is present in amount from about 1 mg/ml to about 50 mg/ml.

39. The process of Claims 37 or 38 wherein said echinocandin compound is present in an amount from about 1 mg/ml to about 30 mg/ml.

40. A process for making a freeze-dried formulation comprising in the following order the steps of:

(i) dissolving into an aqueous solvent an echinocandin compound or echinocandin/carbohydrate complex containing said echinocandin compound in the presence of a pharmaceutically acceptable micelle-forming surfactant to form a solution, wherein said surfactant is present in an amount greater than 1% weight per volume of solution;

(ii) sterile filtering said solution; and

(iii) freeze-drying said solution.

41. The process of Claim 40 further comprising the step of adding one or more bulking agents, buffers, stabilizing agents, tonicity agents, or combinations thereof before step (ii).

42. The process of Claim 40 wherein said micelle-forming surfactant is selected from the group consisting of polysorbates, polyoxyethylene castor oil derivatives, polyoxyethylene stearates, sorbitan trioleate, bile salts, lecithin and combinations thereof.

43. A process for preparing a freeze-dried formulation comprising the steps of

(i) buffering a non-toxic aqueous solvent to a pH between 4.0 and 5.5 to form a buffered solution;

(ii) adding to said buffered solution a pharmaceutically acceptable, micelle-forming surfactant;

(iii) cooling the solution from step (ii) to a temperature between 5° and 15°C to form a cooled solution;

(iv) adding a slurry comprising an echinocandin compound or echinocandin/carbohydrate complex and a second non-toxic aqueous solvent to said cooled solution;

(v) sterile filtering said solution from step (iv); and

(vi) freeze-drying said solution from step (v).

44. The process of Claim 43 wherein said temperature in step (iii) is from about 7°C to about 10°C.

45. The process of Claim 43 further comprising the step of adding one or more bulking agents, stabilizing agents, tonicity agents, or combinations thereof before step (v).

46. A parenteral formulation comprising an aqueous solvent and a freeze-dried formulation prepared by the process of Claims 40 - 45.

47. A parenteral pharmaceutical product prepared by (i) dissolving into an aqueous solvent an echinocandin compound or echinocandin/carbohydrate complex containing said echinocandin compound in the presence of a pharmaceutically acceptable micelle-forming surfactant to form a solution, wherein said surfactant is present in an amount greater than 1% weight per volume of solution; (ii) sterile filtering said solution; and (iii) freeze-drying said solution from step (ii) in a vial.

48. The product of Claim 47 wherein the preparation of said product further comprising adding a non-toxic, aqueous solvent to said vial after step (iii).

49. The product of Claim 47 wherein the weight ratio of echinocandin compound to surfactant is from about 1:1.75 to about 1:25.

50. A method of treating an antifungal infection in a mammal in need thereof comprising the step of administering to said mammal a parenteral formulation of Claim 1.

51. A method of treating an antifungal infection in a mammal in need thereof comprising the step of administering to said mammal a parenteral formulation of Claim 31.

52. A method of treating an antifungal infection in a mammal in need thereof comprising the step of administering to said mammal a parenteral formulation of Claim 46.

53. The formulation of Claim 1 wherein said bulking agent is selected from the group consisting of mannitol, sucrose, trehalose, lactose and mixtures thereof.