

5 COMPOSITIONS AND METHODS FOR PREVENTING AND TREATING
 ENDOTOXIN-RELATED DISEASES AND CONDITIONS

Background of the Invention

 This invention relates to compositions and methods for preventing and
10 treating endotoxin-related diseases and conditions.

 Since the 1930's, the increasing use of immunosuppressive therapy and
invasive devices, as well as the increased incidence of antibiotic resistance in bacteria,
have led to a gradual rise in the occurrence of sepsis and septic shock. Currently, the
estimated incidences in the United States of sepsis and septic shock are 400,000 and
15 200,000 patients/year, respectively. This results in about 100,000 fatalities/year,
making septic shock the most common non-coronary cause of death in the hospital
Intensive Care Unit (ICU). Currently, ICU therapy for septic shock generally involves
treatment with antibiotics, cardiovascular resuscitation, vasopressor/ionotrope therapy,
and/or ventilatory support. This ICU care can cost up to \$1,500/day/patient, resulting
20 in an average total cost per patient of \$13,000 to \$30,000, due to the typical length of
ICU stay.

 Sepsis and septic shock are caused by the release of a molecule known as
endotoxin or lipopolysaccharide (LPS) from the walls of growing and dying gram-
negative bacteria. The released endotoxin induces many pathophysiological events,
25 such as fever, shock, disseminated intravascular coagulation (DIC), and hypotension,
in infected patients. Medicines for the treatment of gram-negative sepsis have been
desired for some time, especially drugs that block endotoxin or cytokines induced by
endotoxin-mediated cellular stimulation. To this end, various strategies for treatment
have included administration of antibodies or other agents against LPS, or cytokines,
30 such as TNF- α and interleukin-1. For various reasons, these approaches have failed.

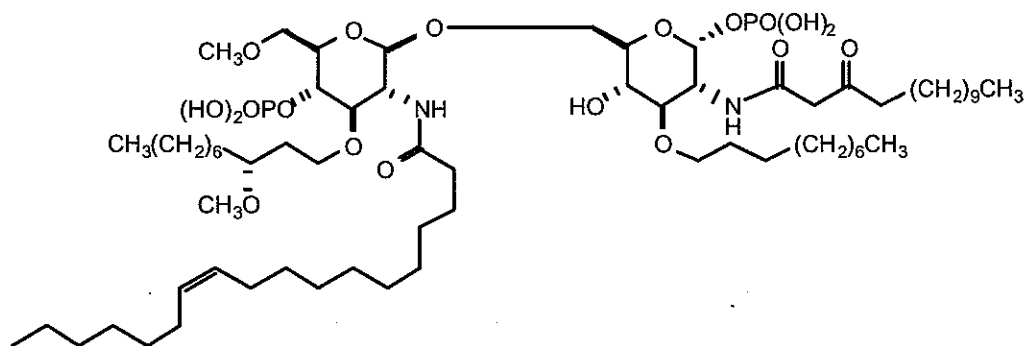
 While endotoxin itself is a highly heterogenous molecule, the toxic
properties of endotoxin are attributable to the highly conserved hydrophobic lipid A
portion of the molecule. An effective drug that acts as an antagonist to this conserved

structure is known as E5564 (also known as compound 1287 and SGEA). This drug is described as compound 1 in U.S. Patent No. 5,935,938, which is incorporated herein by reference.

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Summary of the Invention

In one aspect of the present invention, there is provided an aqueous pharmaceutical composition comprising a compound having the formula (I):



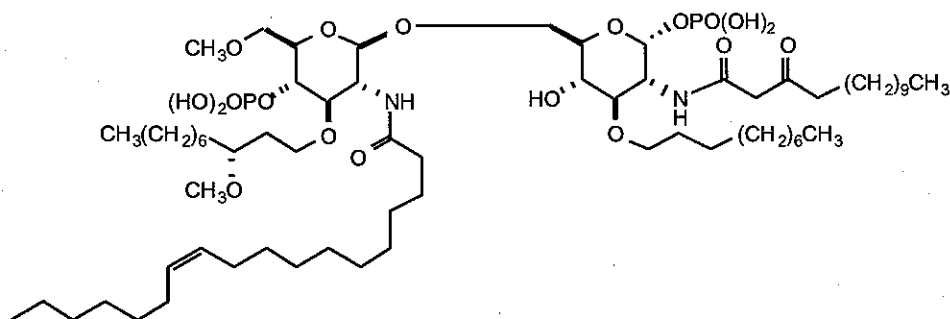
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(I)

or pharmaceutically acceptable salt thereof, and an antioxidant and a disaccharide stabilizing agent.

The disclosure herein provides compositions including the antiendotoxin compound E5564, which has the formula:

15



, or

pharmaceutically acceptable salts thereof, and an antioxidant.

Examples of antioxidants that can be used in the compositions disclosed herein include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, sodium sulfite, sodium thiosulfate, monothioglycerol, tert-butyl hydroquinone, ethoxyquin, dithiothreitol, and derivatives thereof. The compositions of the invention can also include disaccharide stabilizing agents (e.g., a disaccharide such as lactose, sucrose, trehalose, or maltose) and/or include sodium ions in amounts of 0.5-10 mM or ≤ 2 mM, so as to stabilize the micelle size of the antiendotoxin compound at about 7-9 nm during lyophilization.

The disclosure herein also provides methods of making pharmaceutical compositions including antiendotoxin compounds, which involve admixing the compounds with an antioxidant. An example of an antiendotoxin compound that can be included in these compositions is E5564 (or pharmaceutically acceptable salts thereof). Examples of antioxidants present in these compositions include butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, sodium sulfite, sodium thiosulfate, monothioglycerol, tert-butyl hydroquinone, ethoxyquin, dithiothreitol, and derivatives thereof.

Also included herein are methods of making pharmaceutical compositions including antiendotoxin compounds. These methods can include the steps of: (i) dissolving the antiendotoxin compound in an aqueous solution of sodium hydroxide; (ii) adding a disaccharide stabilizer (e.g., lactose) to the solution; (iii) adding an antioxidant (e.g., butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, sodium sulfite, sodium thiosulfate, monothioglycerol, tert-butyl hydroquinone, ethoxyquin, dithiothreitol, or a derivative thereof) to the solution; (iv) lowering the pH of the solution (to, e.g., about pH 7 - 8, by use of, e.g., a phosphoric acid solution); (v) filter sterilizing the solution; and (vi) freeze-drying the solution (using, e.g., a process including the use of a shelf temperature of 0°C - 20°C).

The disclosure herein also includes methods of preventing or treating endotoxemia in patients, involving administration of the pharmaceutical compositions described herein to the patients, as well as use of the compositions described herein in the prevention and treatment of endotoxemia.

In another aspect of the present invention, there is provided a use of the composition of the invention in the manufacture of a medicament for prevention or treatment of endotoxemia.

In another aspect of the present invention, there is provided a lyophilized pharmaceutical composition, said lyophilized pharmaceutical composition comprising the lyophilisate of an aqueous solution, said aqueous solution comprising:

about 0.26% (weight/volume) of the compound of formula (I);

about 9.7% (weight/volume) lactose monohydrate;

about 0.024% (weight/volume) of 0.15M phosphoric acid;

about 0.00017% (weight/volume) of butylated hydroxyanisole;

about 0.0073% (weight/volume) of 5mM sodium hydroxide; and

about 90.0% (weight/volume) water for injection;

wherein sodium ions are present at a concentration of $\leq 2\text{mM}$, provided that when said compound of formula (I) is provided as a sodium-containing salt, said aqueous solution comprises sodium ions in an amount of about 2 mM, excluding sodium ions contributed to said aqueous solution by said sodium salt of formula (I).

The disclosure herein provides several advantages. The discoveries described herein with respect to formulation yield a drug product having increased stability, without any sacrifice in drug quality. For example, by including antioxidants, the compositions disclosed are stable to oxidative degradation. In addition, the inclusion of disaccharides and the use of only low amounts of sodium ions enables the maintenance of micelle size throughout the freeze-drying process. Further, the freeze-drying process employed in making the compositions disclosed includes the use of a relatively high shelf temperature, which results in a more efficient formulation process.

In another aspect, there is provide a use of the composition as defined above, in producing a medicament for preventing or treating a disease or condition associated with sepsis, septic shock, or endotoxemia in a patient.

Other features and advantages of the invention will be apparent from the following detailed description, the drawings, and the claims.

Brief Description of the Drawings

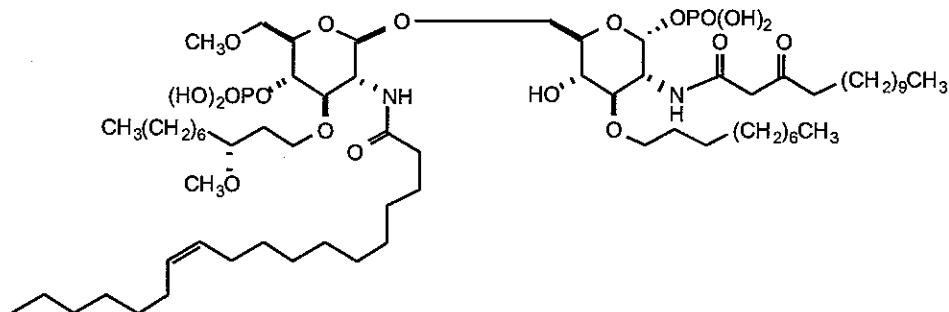
Figure 1 is a graph showing the relative stability of E5564 in aqueous solution prepared with different drug substance lots, as measured by the formation of major oxidative degradants. The graph shows the HPLC peak area percent of major oxidative degradants present over time for four drug substance lots: 1 (◆), 2 (■), 3 (▲), and 4 (●), the latter of which includes 7 µg BHA/vial.

Figure 2 is a flowchart showing a manufacturing scheme for E5564.

Detailed Description

The invention provides pharmaceutical compositions that include an antiendotoxin compound, as well as methods of preparing and using such compositions. The invention is based on the discovery that certain formulation components and steps are particularly advantageous in terms of the quality of the drug product and/or the efficiency of the formulation process. The details of the pharmaceutical compositions of the invention, as well as methods of their production and use, are provided below.

An example of an antiendotoxin compound that can be included in the compositions of the invention is E5564, which has the formula:



, or

pharmaceutically acceptable salts of this compound. E5564 can be made by using, for example, the synthetic methods described in U.S. Patent No. 5,935,938, and may be subjected to further purification steps, for example, the purification methods described in international application PCT/US02/16203 (WO 02/094019 A1). Additional examples of antiendotoxin compounds that can be included in the compositions of the invention include compound B531 (U.S. Patent No. 5,530,113), as well as other antiendotoxin compounds described in these patents and the following U.S. patents:

U.S. Patent No. 5,612,476, U.S. Patent No. 5,756,718, U.S. Patent No. 5,843,918, U.S. Patent No. 5,750,664, and U.S. Patent No. 5,681,824, the teachings of which are incorporated herein by reference.

The pharmaceutical compositions of the invention can also include, in addition to an antiendotoxin compound, components that we have discovered as providing beneficial features to the compositions. For example, the compositions of the invention can include an antioxidant compound, as we have found that such compounds render the drug product solution stable to degradation by oxidation, without having any adverse effects on drug product quality. An example of an antioxidant compound that can be included in the pharmaceutical compositions of the invention is butylated hydroxyanisole (BHA). Additional examples of antioxidant compounds that can be included in the compositions of the invention are butylated hydroxytoluene (BHT), propyl gallate, sodium sulfite, sodium thiosulfate, monothioglycerol, tert-butyl hydroquinone, ethoxyquin, dithiothreitol, and other antioxidant compounds that are known in the art. Appropriate amounts of these compounds to be included in the compositions of the invention can readily be determined by those of skill in this art, using as guidance, for example, the teachings herein. For example, BHA can be present in the compositions of the invention in amounts ranging from, for example, 0.5-100, 1-50, 2-25, or 5-15 $\mu\text{g}/10\text{ mg}$ drug. As a specific example, we note that 7.2 μg BHA is used in a formulation of 10 mg of E5564 that is described in further detail below.

As is discussed further below, we have also found that including a disaccharide, such as lactose, in the compositions of the invention improves the quality of these compositions. In particular, amphiphilic molecules, such as E5564, self associate into micelles in aqueous solution. We have found that including a disaccharide in the compositions of the invention stabilizes the size of the E5564 micelles during lyophilization, as is described in further detail below. Use of disaccharides in the drug formulation process thus facilitates consistency in this process. In addition to lactose, other disaccharides can be included in the compositions of the invention. For example, sucrose, trehalose, or maltose can be used. These compounds can be present in amounts determined to be appropriate by

those of skill in this art. For example, they can be present in amounts ranging from 1-20% or 5-15% weight/volume. As a specific example, we note that a formulation of 10 mg of E5564 that is described in further detail below includes 9.7% lactose. Use of this amount provides for good maintenance of micelle size.

5 We have also found that the ionic strength of the drug solution impacts the stability of the micelle size of the drug during lyophilization. In particular, we have found that minimizing the amount of sodium ions in the drug solution leads to greater stability of the micelle size. In previous methods, sodium phosphate salts had been used to lower the pH of the alkaline solution in which the drug is initially dissolved
10 (see below). We have found that use of phosphoric acid for this purpose can minimize the amount of sodium ions in the formulation, and result in a more stable product. The formulations of the invention can thus include 1-15, e.g., 2-10, mM Na⁺ (or K⁺) (excluding consideration of Na⁺ contributed from the drug). Thus, the compositions of the invention can include, for example, 10 mM Na⁺ (or K⁺) or less,
15 such as, for example, 5, 4, 3, 2, or 1 mM Na⁺ (excluding Na⁺ from the drug)(or K⁺). As a specific example, we note that use of 2 mM Na⁺ (excluding consideration of Na⁺ contributed from the drug) in formulating E5564 results in good stability of micelle size, as is described in further detail below.

Also included in the invention is our discovery of a freeze-drying approach
20 that facilitates more efficient formulation of the drug product, while not adversely affecting product quality. As is discussed further below, the compositions of the invention can be made by using a process including the following steps. First, the drug is dissolved in a dilute, aqueous NaOH solution at pH 10.1-11.8, which facilitates dissolution and dispersion of E5564 into micelles of uniform size. The
25 alkaline E5564 solution is then combined with a lactose solution and a solution including an antioxidant. A phosphoric acid solution is used to neutralize the solution to a pH of about 7.0 - 8.0. The solution is then adjusted to a target volume with water, filter sterilized, aseptically filled into glass vials, and freeze-dried to render the product stable for long-term storage. As is well known in the art, low shelf
30 temperatures (e.g., -25°C) are typically used to keep product temperature low, to avoid product collapse during freeze-drying (see, e.g., Pikal, Intl. J. Pharm. 62:165-186,

1990). Surprisingly, we have found that use of relatively high shelf temperatures (e.g., +20°C) results in good quality product (no collapse). Thus, the invention includes the use of relatively high shelf temperatures (e.g., 0°C - 20°C) in the freeze-drying process.

5 The invention also includes methods of making pharmaceutical compositions that include an antiendotoxin compound and an antioxidant, as described herein. These methods include the steps outlined above, i.e, dissolving the drug in a basic solution (e.g., NaOH), addition of a disaccharide stabilizer, addition of an antioxidant (e.g., BHA or any of the other antioxidants listed above), addition of an
10 acidic solution (e.g., phosphoric acid) to lower the pH to 7 - 8, filtration, and freeze-drying. Detailed examples of each of these steps are provided below. Additional appropriate variations of particular steps in the formulation of E5564 can readily be determined by those of skill in this art (see, e.g., *Remington's Pharmaceutical Sciences* (18th edition), ed. A. Gennaro, 1990, Mack Publishing Company, Easton,
15 PA).

 The following is a detailed description of an example of a method for formulating E5564. As is discussed further below, key features of this method include the following. A small amount (7 µg/vial) of butylated hydroxyanisole (BHA) was added to the formulation to prevent oxidation of the E5564 in aqueous solution. As is
20 shown in Figure 1, inclusion of BHA in the drug product formulation increases its stability in solution. In addition, the sodium content of the solution was lowered by replacing sodium phosphate salts with phosphoric acid for pH adjustment, to enhance micelle size stability during the freeze-drying manufacturing step, and a high shelf temperature was used in the freeze-drying step of the process.

25 The table below shows the components and composition of an E5564 10 mg vial that is manufactured using the steps set forth below. Also shown in the table is an indication of the function of each component.

Composition of dosage form

Strength (Label claim):	10 mg vial		
Component and Quality Standard (and Grade, if applicable)	Quantity per unit	%	Function of component
E5564 Drug Substance, Eisai Standard	10.0 mg as free acid, 10.7 mg as tetrasodium salt	0.26%	Active ingredient
Lactose Monohydrate, NF	400 mg	9.7%	Bulking agent
Phosphoric Acid, NF ^A	0.98 mg	0.024%	pH adjustment
Butylated hydroxyanisole, NF ^C	0.0072 mg	0.00017%	Antioxidant
Sodium Hydroxide, NF ^A	0.30 mg	0.0073%	pH adjustment
Water for Injection, USP ^B	3724 mg	90.0%	Solvent

^A Used for pH adjustment, quantity will vary with lot.

^B Water for Injection (WFI) is removed during freeze-drying. Quantity will vary with lot.

^C A 10% overage is used to account for manufacturing losses.

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Description of a Representative Manufacturing Process

Formulation Compounding

- 1) Prepare a 5mM NaOH solution
- 2) Prepare drug solution by accurately weighing and dissolving E5564 drug substance in NaOH solution at 20°C - 60°C. The pH of the E5564/alkaline solution is pH 10.1 – 12.0 after dissolution of E5564.
- 3) Prepare a 0.15M phosphoric acid solution.
- 4) Preparation a BHA solution.
- 5) Prepare a lactose solution.
- 6) Mix the lactose and the drug solutions. Add the phosphoric acid and the BHA solutions and add water to yield the target formulation concentrations.

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Filtration

Filter the formulated solution using a Pall Kleenpak Ultipor[®] N66[®] nylon filter with a pore size of 0.2µm.

5 Filling and Semi-Plugging

Fill the formulated solution into vials and partially seat lyophilization closures in the vials. Transfer the vials to a freeze-dryer.

Freeze-Drying

10 Freeze-dry the filled vials under the following conditions:

- 1) Vials are loaded at +20°C and then the shelf temperature is lowered to ≤ -40°C.
- 2) The product is held at -40°C for 3 hours after reaching steady state.
- 3) Primary drying occurs at shelf temperatures of +20°C (see below for use of
15 other shelf-temperatures).
- 4) Secondary drying occurs at shelf temperatures of +20 to +25°C.
- 5) Pre-aerate the chamber with nitrogen or air.
- 6) Fully seat the lyophilization closures.

20 Sealing

Seal the vials with aluminum caps.

The following section describes experiments carried out to determine the impact of certain variations in formulation parameters on the quality of product formation.

25

Method of Freeze-drying E5564 drug product

We discovered that a relatively high shelf temperature (e.g., +20°C) could be used during primary drying to freeze-dry the lactose-containing E5564 drug product. This results in a more efficient manufacturing process, as compared to a conventional low shelf temperature (e.g., -25°C). Low shelf-temperatures are typically used to keep product temperatures low, to avoid product collapse during freeze-drying (see, e.g., Pikal, Intl. J. Pharm. 62:165-186, 1990). Product collapsed when freeze-dried with Cycle B (see table below), which used a linear increase in shelf temperature from -40°C to +20°C at +3°C/ hour. It was surprising to discover that Cycles C and D resulted in a good quality product (no collapse), as the shelf temperatures in these cycles were greater than that of Cycle B. Based on these results, we employ a shelf-temperature of 0 to +20°C with a chamber pressure of <0.1 mmHg. In addition, chamber pressure can be maintained at <0.075 mmHg.

Cycle	ST (°C)	P (mmHg)	PT (°C)	Collapse
A	-25	0.1	-31	no
B	- 40 to +20	0.1	-31 to -24	yes
C	+20	0.1	-27	no
D	+20	0.02	-28	no

ST = shelf temperature
P = chamber pressure
PT = product temperature

Importance of disaccharides to formulation

We discovered that disaccharides are useful for preparing freeze-dried preparations of the drug. We have shown that lactose and sucrose are effective at stabilizing the micelle size during freeze-drying (see data in the table set forth below).

The micelle size before freeze-drying was 7 nm.

Stabilization of Micelle Size During Lyophilization of E5564 as a Function of Lactose Concentration

%(w/v) Lactose in Formulation ^A	Micelle Size (hydrodynamic diameter)
1%	16 nm
2%	13 nm
5%	8 nm
10%	7 nm

^A The micelle size before freeze-drying was 7 nm

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Importance of low ionic strength to formulation

We discovered that minimization of the salt concentration in the formulation is important in maintaining the desired micelle size during freeze-drying. A formulation containing 10 mM Na⁺ (excluding the sodium contributed from the drug) works for some, but not all, lots of E5564. Thus, we employ formulations including 2 mM Na⁺ (excluding the Na⁺ contributed from the drug). Similar parameters apply with respect to potassium ion (K⁺) concentrations.

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Importance of Antioxidant to Formulation

We tested several antioxidant compounds to determine whether they impact the stability of E5564 to free-radical oxidation. The table set forth below summarizes the antioxidants tested. We have also found dithiothreitol to be effective.

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Antioxidant Screening Experiments

Antioxidant	time	Major Oxidative Degradant	E5564 conc. (% initial)	Comments
none	0 hours	1.12	100	Control
none	18-22 hours	6.02	91.3	Rapid degradation
BHT	0 hours	0.77	100	
BHT	18-22 hours	0.24	102.8	Effective
BHA	0 hours	0.68	100	
BHA	18-22 hours	0.27	102.3	Effective
Propyl Gallate	0 hours	0.38	100	
Propyl Gallate	18-22 hours	0.32	100.6	Effective
Vitamin E Acetate	0-4 hours	1.34	100	
Vitamin E Acetate	18-22 hours	4.55	94.2	Not effective
Ascorbic Acid	0-4 hours	0.08	100	
Ascorbic Acid	18-22 hours	0.11	97.4	Not useful , stabilizing to oxidation but E5564-Ascorbic acid react forming new impurities
Ascorbyl Palmitate	0-4 hours	0.15	100	
Ascorbyl Palmitate	18-22 hours	0.19	92.4	Not useful , stabilizing to oxidation but E5564-Ascorbyl palmitate react forming new impurities
Sodium Sulfite	0-4 hours	0.13	100	
Sodium Sulfite	18-22 hours	0.25	101.8	Effective
Sodium Thiosulfate	0-4 hours	0.09	100	
Sodium Thiosulfate	18-22 hours	0.19	100.2	Effective
Monothioglycerol	0-4 hours	0.13	100	
Monothioglycerol	18-22 hours	0.16	101.3	Effective

Use of the Compositions of the Invention

The compositions of the invention can be used to prevent or to treat any of a large number of diseases and conditions associated with sepsis, septic shock, or endotoxemia. For example, the compositions and methods of the invention can be
5 used in conjunction with any type of surgery or medical procedure, when appropriate, that could lead to the occurrence of endotoxemia or related complications (e.g., sepsis syndrome). As a specific example, the invention can be used in conjunction with cardiac surgery (e.g., coronary artery bypass graft, cardiopulmonary bypass, and/or valve replacement), transplantation (of, e.g., liver, heart, kidney, or bone marrow),
10 cancer surgery (e.g., removal of a tumor), or any abdominal surgery (see, e.g., PCT/US01/01273).

Additional examples of surgical procedures with which the compositions and methods of the invention can be used, when appropriate, are surgery for treating acute pancreatitis, inflammatory bowel disease, placement of a transjugular
15 intrahepatic portosystemic stent shunt, hepatic resection, burn wound revision, and burn wound escharectomy. The compositions of the invention can also be used in conjunction with non-surgical procedures in which the gastrointestinal tract is compromised. For example, the compositions can be used in association with chemotherapy or radiation therapy in the treatment of cancer. The compositions and
20 methods of the invention can also be used in the treatment of conditions associated with HIV infection, trauma, or respiratory distress syndrome, as well as with immunological disorders, such as graft-versus-host disease or allograft rejection. Pulmonary bacterial infection and pulmonary symptomatic exposure to endotoxin can also be treated using the compositions and methods of the invention (see, e.g.,
25 PCT/US00/02173).

Administration of the compositions of the invention can be carried out using any of several standard methods including, for example, continuous infusion, bolus injection, intermittent infusion, inhalation, or combinations of these methods. For example, one mode of administration that can be used involves continuous
30 intravenous infusion. In such an approach, the infusion dosage rate of the drug can be, for example, 0.001-0.5 mg/kg body weight/hour, more preferably 0.01-0.2

mg/kg/hour, and most preferably 0.03-0.1 mg/kg/hour, with the drug being infused over the course of, for example, 12-100, 60-80, or about 96 hours. The infusion of the drug can, if desired, be preceded by a bolus injection; preferably, such a bolus injection is given at a dosage of 0.001-0.5 mg/kg. Preferably, the total amount of drug administered to a patient is 25-600 mg of drug, more preferably 35-125 mg, by infusion over a period of 60-100 hours. As activity in the hospital, and particularly the ICU, is often hectic, minor variations in the time period of infusion of the drugs may occur and are also included in the invention.

Additional modes of administration of E5564, according to the methods of the invention, include bolus or intermittent infusion. For example, the drug can be administered in a single bolus by intravenous infusion through, for example, a central access line or a peripheral venous line, or by direct injection, using a syringe. Such administration may be desirable if a patient is only at short-term risk for exposure to endotoxin, and thus does not need prolonged persistence of the drug. For example, this mode of administration may be desirable in surgical patients, if appropriate, such as patients having cardiac surgery, e.g., coronary artery bypass graft surgery and/or valve replacement surgery. In these patients, a single bolus infusion of, e.g., 0.10-15 mg/hour (e.g., 1-7 mg/hour or 3 mg/hour) of drug can be administered over a period of four hours prior to and/or during surgery. (Note that the amount of drug administered is based on an assumed average weight of a patient of 70 kg.) Shorter or longer time periods of administration can be used, as determined to be appropriate by one of skill in this art.

In cases in which longer-term persistence of active drug is desirable, for example, in the treatment of a condition associated with long-term exposure to endotoxin, such as during infection or sepsis, or in appropriate surgical situations in which it is determined that prolonged treatment is desirable, intermittent administration can be carried out. In these methods, a loading dose is administered, followed by either (i) a second loading dose and a maintenance dose (or doses), or (ii) a maintenance dose or doses, without a second loading dose, as determined to be appropriate by one of skill in this art.

The first (or only) loading dose can be administered in a manner similar to that described for the single bolus infusion described above. That is, for E5564 administration, 0.10-15 mg/hour (e.g., 3-7 mg/hour or 3 mg/hour) of drug can be administered to a patient over a period of four hours prior to surgery. If a second
5 loading dosage is to be used, it can be administered about 12 hours after the initial loading dose and can involve infusion of, e.g., 0.10-15 mg/hour (e.g., 1-7 mg/hour or 3 mg/hour) of drug over a period of, e.g., about two hours.

To achieve further persistence of active drug, a maintenance dose (or doses) of drug can be administered, so that levels of active drug are maintained in the
10 blood of a patient. Maintenance doses can be administered at levels that are less than the loading dose(s), for example, at a level that is about 1/6 of the loading dose. Specific amounts to be administered in maintenance doses can be determined by a medical professional, with the goal that drug level is at least maintained. Maintenance doses can be administered, for example, for about 2 hours every 12 hours beginning at
15 hour 24 and continuing at, for example, hours 36, 48, 60, 72, 84, 96, 108, and 120. Of course, maintenance doses can be stopped at any point during this time frame, as determined to be appropriate by a medical professional.

The infusion methods described above can be carried out using catheters (e.g., peripheral venous, central venous, or pulmonary artery catheters) and related
20 products (e.g., infusion pumps and tubing) that are widely available in the art. One criterion that is important to consider in selecting a catheter and/or tubing to use in these methods is the impact of the material of these products (or coatings on these products) on the micelle size of the drug. In particular, we have found that the use of certain products generally maintains a drug micelle size of 7-9 nm. Examples of such
25 catheters are the following Baxter (Edwards) catheters: Swan-Ganz, VANTEX, Multi Med, and AVA Device. Additional examples of catheters that can be used for this purpose are the Becton-Dickinson Criticath catheter; the Arrow International multi-lumen, Arrowg+ard Blue, and Large-bore catheters; and the Johnson & Johnson Protectiv I.V. catheter.

Additional catheter-related products that can be used in the methods of the invention can be identified by determining whether the material of the products alters micelle size of the drug, under conditions consistent with those that are used in drug administration. In addition, in the event that a patient already has a catheter in place
5 that does not maintain optimal drug micelle size, a catheter insert that is made of a compatible material (e.g., a polyamide polymer) or that includes a compatible coating can be used so that the drug solution does not contact the surface of the incompatible catheter. Such an insert, having an outside diameter that is small enough to enable it to be easily inserted into the existing catheter, while maintaining an inside diameter
10 that is large enough to accommodate drug solution flow, is placed within the existing catheter and connected to tubing or a syringe through which the drug is delivered.

In the case of pulmonary bacterial infection or pulmonary symptomatic exposure to endotoxin, administration of the compositions of the invention can be effected by means of periodic bolus administration, by continuous, metered inhalation,
15 or by a combination of the two. A single dose is administered by inhalation 1 μ g-24 mg, for example, 5-150 μ g, or, preferably, 10-100 μ g of the drug. Of course, recalcitrant disease may require administration of relatively high doses, e.g., 5 mg, the appropriate amounts of which can be determined by one of skill in this art. Appropriate frequency of administration can be determined by one of skill in this art
20 and can be, for example, 1-4, for example, 2-3, times each day. Preferably, the drug is administered once each day. In the case of acute administration, treatment is typically carried out for periods of hours or days, while chronic treatment can be carried out for weeks, months, or even years.

Both chronic and acute administration can employ standard pulmonary
25 drug administration formulations, which can be made from the formulations described elsewhere herein. Administration by this route offers several advantages, for example, rapid onset of action by administering the drug to the desired site of action, at higher local concentrations. Pulmonary drug formulations are generally categorized as nebulized (see, e.g., Flament et al., *Drug Development and Industrial Pharmacy*
30 21(20):2263-2285, 1995) and aerosolized (Sciarra, "Aerosols," Chapter 92 in *Remington's Pharmaceutical Sciences*, 16th edition (ed. A. Osol), pp. 1614-1628;

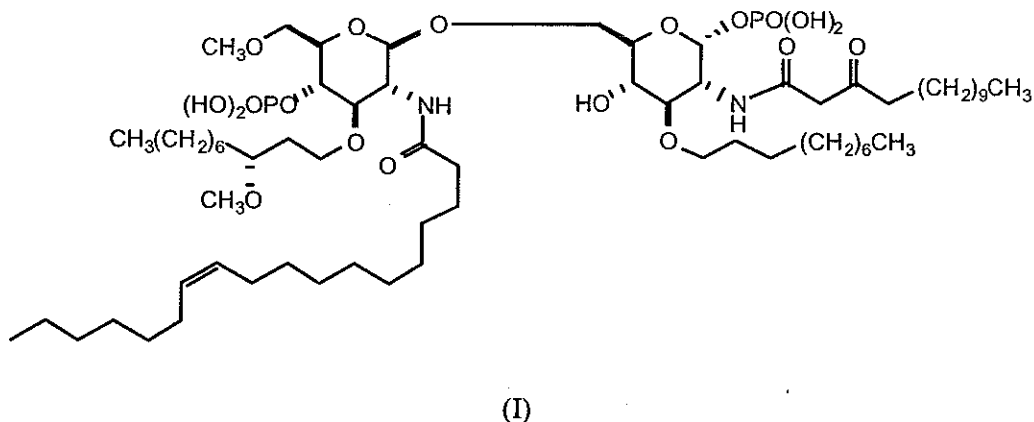
Malcolmson et al., PSTT 1(9):394-398, 1998, and Newman et al., "Development of New Inhalers for Aerosol Therapy," in Proceedings of the Second International Conference on the Pharmaceutical Aerosol, pp. 1-20) formulations.

All patents and publications mentioned herein are incorporated by
5 reference.

Other embodiments are within the following claims.

Claims:

1. An aqueous pharmaceutical composition comprising a compound having formula (I):



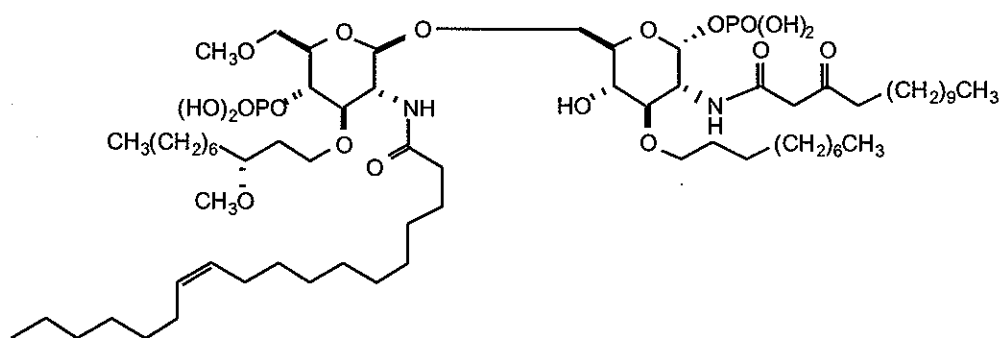
or pharmaceutically acceptable salt thereof, and an antioxidant and a disaccharide stabilizing agent.

2. The composition of claim 1, wherein said antioxidant is selected from the group consisting of butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, sodium sulfite, sodium thiosulfate, monothioglycerol, tert-butyl hydroquinone, ethoxyquin, dithiothreitol, and derivatives thereof.
3. The composition of claim 2, wherein said antioxidant is selected from the group consisting of butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, sodium sulfite, sodium thiosulfate, monothioglycerol, and dithiothreitol.
4. The composition of claim 3, wherein said antioxidant is butylated hydroxyanisole.
5. The composition of claim 4, wherein said butylated hydroxyanisole is present in an amount ranging from 0.5 to 100 micrograms per about 10 mg of the compound of formula (I).
6. The composition of claim 5, wherein said butylated hydroxyanisole is present in an amount ranging from 1 to 50 micrograms per about 10 mg of the compound of formula (I).
7. The composition of claim 6, wherein said butylated hydroxyanisole is present in an amount ranging from 2 to 25 micrograms per about 10 mg of the compound of formula (I).
8. The composition of claim 7, wherein said butylated hydroxyanisole is present in an amount ranging from 5 to 15 micrograms per about 10 mg of the compound of formula (I).

9. The composition of claim 8, wherein said butylated hydroxyanisole is present in an amount of about 7 micrograms per about 10 mg of the compound of formula (I).
10. The composition according to any one of claims 1-10, wherein said disaccharide stabilizing agent is lactose, sucrose, trehalose or maltose.
- 5 11. The composition of claim 10, wherein said disaccharide is trehalose or maltose.
12. The composition of claim 10, wherein said disaccharide is lactose.
13. The composition of claim 10, wherein said disaccharide is sucrose.

14. The composition according to claim 10, wherein said disaccharide is present in an amount ranging from 1% to 20% (weight/volume).
15. The composition according to claim 14, wherein said disaccharide is present in an amount ranging from 5% to 15% (weight/volume).
- 5 16. The composition according to claim 15, wherein said disaccharide is present in an amount of about 10% (weight/volume).
17. The composition according to claim 16, wherein said disaccharide is lactose.
18. The composition of claim 1, wherein said composition comprises one or more monovalent metal ions selected from the group consisting of sodium and potassium;
- 10 wherein said monovalent metal ions are present in an amount ranging from 0.5 mM to 15 mM, wherein when the compound of formula (I) is provided as a sodium-containing and/or a potassium-containing salt, said amount of said monovalent metal ions refers to the amount of said monovalent metal ions present exclusive of the sodium and/or potassium ions contributed to said composition by said salt.
- 15 19. The composition of claim 18, wherein said composition comprises sodium ions in an amount ranging from 0.5 mM to 10 mM.
20. The composition of claim 19, wherein said monovalent metal ions are present in an amount ranging from 2 mM to 10 mM.
21. The composition of claim 20, wherein said monovalent metal ions are present in
- 20 an amount of about 5 mM, about 4 mM, about 3 mM, about 2 mM, or about 1 mM.

22. The composition of claim 18, wherein said composition comprises sodium ions in an amount of ≤ 2 mM.
23. The composition of claim 1, wherein said compound of formula (I) is provided in the form of micelles.
24. The composition of claim 23, wherein the micelle size ranges from 7 nm to 16 nm.
25. The composition of claim 23, wherein the micelle size of said compound is about 7-9 nm.
26. A method of making a pharmaceutical composition comprising an antiendotoxin compound, said method comprising admixing said antiendotoxin compound and an antioxidant.
27. The method of claim 26, wherein said antiendotoxin compound is a compound having formula (I):



(I)

or pharmaceutically acceptable salt thereof.

28. The method of claim 26, wherein said antioxidant is selected from the group consisting of butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, sodium sulfite, sodium thiosulfate, monothioglycerol, tert-butyl hydroquinone, ethoxyquin, dithiothreitol, and derivatives thereof.

29. The method of claim 28, wherein said antioxidant is selected from the group consisting of butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, sodium sulfite, sodium thiosulfate, monothioglycerol, and dithiothreitol.
30. The method of claim 29, wherein said antioxidant is butylated hydroxyanisole.
- 5 31. A method of making a pharmaceutical composition comprising an antiendotoxin compound, said method comprising the steps of:
- (i) dissolving said antiendotoxin compound in an aqueous solution of sodium hydroxide;
 - (ii) adding a disaccharide stabilizer to said solution;
 - 10 (iii) adding an antioxidant to said solution;
 - (iv) lowering the pH of said solution;
 - (v) filter sterilizing said solution; and
 - (vi) freeze-drying said solution.
- 15 32. The method of claim 31, wherein said disaccharide is lactose, sucrose, trehalose or maltose.
33. The method of claim 32, wherein said disaccharide is lactose.
34. The method of claim 32, wherein said disaccharide is sucrose.
- 20 35. The method of claim 31, wherein said antioxidant is selected from the group consisting of butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, sodium sulfite, sodium thiosulfate, monothioglycerol, tert-butyl hydroquinone, ethoxyquin, dithiothreitol, and derivatives thereof.
36. The method of claim 35, wherein said antioxidant is selected from the group consisting of butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, sodium sulfite, sodium thiosulfate, monothioglycerol, and dithiothreitol.
- 25 37. The method of claim 36, wherein said antioxidant is butylated hydroxyanisole.

38. The method of claim 31, wherein said pH of said solution is lowered to about pH 7 - 8, using a phosphoric acid solution.
39. The method of claim 31, wherein said antiendotoxin compound is dissolved in a 5 mM aqueous solution of sodium hydroxide at a temperature of 20 °C to 60 °C.
- 5 40. The method of claim 31, wherein said freeze-drying step comprises the use of a shelf temperature in the range of from 0°C to 20°C.
41. The method of claim 21, wherein during said freeze-drying step:
- a) said solution is filled into vials and transferred to a shelf of a freeze-dryer at about 20°C;
 - 10 b) the temperature of said shelf is lowered to $\leq -40^{\circ}\text{C}$, freezing said solution;
 - c) said frozen solution is held at about -40°C for about 3 hours after reaching steady state;
 - d) primary drying occurs at a shelf temperature of about 20 °C; and
 - e) secondary drying occurs at a shelf temperature in the range of from 20°C to 25°C.
- 15 42. The method of claim 41, wherein said primary drying occurs at a pressure of from 0.075 mmHg to 0.1 mmHg.
43. Use of the composition of anyone of claims 1 to 25 in the manufacture of a medicament for prevention or treatment of endotoxemia.
44. The composition of any one of claims 1 to 25 for use in the prevention or treatment of
20 endotoxemia.
45. A lyophilized pharmaceutical composition, said lyophilized pharmaceutical composition comprising the lyophilisate of an aqueous solution, said aqueous solution comprising:
- about 0.26% (weight/volume) of the compound of formula (I);
 - 25 about 9.7% (weight/volume) lactose monohydrate;

about 0.024% (weight/volume) of 0.15M phosphoric acid;

about 0.00017% (weight/volume) of butylated hydroxyanisole;

about 0.0073% (weight/volume) of 5mM sodium hydroxide; and

about 90.0% (weight/volume) water for injection;

wherein sodium ions are present at a concentration of $\leq 2\text{mM}$, provided that when said compound of formula (I) is provided as a sodium-containing salt, said aqueous solution comprises sodium ions in an amount of about 2 mM, excluding sodium ions contributed to said aqueous solution by said sodium salt of formula (I).

46. Use of the composition of any one of claims 1 to 25 in producing a medicament for preventing or treating a disease or condition associated with sepsis, septic shock, or endotoxemia in a patient.
47. The use according to claim 46, wherein said patient is subject to a surgery or a medical procedure, wherein said surgery or medical procedure is associated with the occurrence of endotoxemia or related complications.
48. The use according to claim 47, wherein said medicament is used in conjunction with cardiac surgery, transplantation surgery, cancer surgery, or abominable surgery.
49. The use according to claim 48, wherein said medicament is used in conjunction with coronary artery bypass graft, cardiopulmonary bypass, or valve replacement; transplantation of the liver, heart, kidney, or bone marrow; or removal of a tumor.

50. The use according to claim 47, wherein said medicament is used in conjunction with surgery for treating acute pancreatic, inflammatory bowel disease, placement of a transjugular intrahepatic portosystemic stent shunt, hepatic resection, burn wound revision, or burn wound escharectomy.
51. The use according to claim 47, wherein said medicament is used in conjunction with a non-surgical procedure in which the gastrointestinal tract is compromised.
52. The use according to claim 51, wherein said medicament is used in conjunction with chemotherapy or radiation therapy in the treatment of cancer.
53. The use according to claim 46, wherein said disease or condition is associated with HIV infection, trauma, or respiratory distress syndrome.
54. The use according to claim 46, wherein said disease or condition comprises an immunological disorder.
55. The use according to claim 54, wherein said immunological disorder comprises graft-versus-host disease or allograft rejection.
56. The use according to claim 46, wherein said disease or condition comprises pulmonary bacterial infection or pulmonary symptomatic exposure to endotoxin.
57. The use according to claim 46, wherein said composition is suitable for being administered by continuous infusion, bolus injection, intermittent infusion, or inhalation, or by a combination thereof.
58. The use according to claim 57, wherein said composition is suitable for being

administered by continuous intravenous infusion.

59. The use according to claim 58, wherein said compound of formula (I) is suitable for being administered at an infusion dosage rate of 0.001-0.5 mg/kg body weight/hour.
60. The use according to claim 59, wherein said compound of formula (I) is suitable for being administered at an infusion dosage rate of 0.01-0.2 mg/kg body weight/hour.
61. The use according to claim 60, wherein said compound of formula (I) is suitable for being administered at an infusion dosage rate of 0.03-0.1 mg/kg body weight/hour.
62. The use according to claim 58, wherein said compound of formula (I) is suitable for being infused over the course of 12-100 hours.
63. The use according to claim 62, wherein said compound of formula (I) is suitable for being infused over the course of 60-80 hours.
64. The use according to claim 63, wherein said compound of formula (I) is suitable for being infused over the course of 96 hours.
65. The use according to claim 58, wherein said infusion is preceded by bolus injection.
66. The use according to claim 65, wherein said bolus injection is suitable for being administered at a dosage of 0.001-0.5 mg of said compound of formula (I) per kg of body weight of said patient.

67. The use according to claim 58, wherein the total amount of said compound of formula (I) administered to said patient over a period of 60-100 hours is 25-600 mg.
68. The use according to claim 67, wherein the total amount of said compound of formula (I) administered to said patient over a period of 60-100 hours is 35-125 mg.
69. The use according to claim 57, wherein said composition is suitable for being administered by bolus or intermittent infusion.
70. The use according to claim 69, wherein said composition is suitable for being administered by bolus intravenous infusion through a central access line or a peripheral venous line.
71. The use according to claim 69, wherein said composition is suitable for being administered by direct injection using a syringe.
72. The use according to claim 69, wherein said composition is suitable for being administered to a surgical patient.
73. The use according to claim 72, wherein said composition is suitable for being administered as a single bolus infusion over a period of four hours prior to and/or during surgery.
74. The use according to claim 73, wherein said composition is suitable for being administered at a dosage rate of 0.10-15 mg/hour of said compound of formula (I) per 70 kg of body weight of said patient.

75. The use according to claim 74, wherein said composition is suitable for being administered at a dosage rate of 1-7 mg/hour of said compound of formula (I) per 70 kg of body weight of said patient.
76. The use according to claim 75, wherein said composition is suitable for being administered at a dosage rate of 3 mg/hour of said compound of formula (I) per 70 kg of body weight of said patient.
77. The use according to claim 57, wherein said composition is suitable for being administered by intermittent infusion.
78. The use according to claim 77, wherein said intermittent infusion comprises:
administering a loading dose to said patient; and
administering one or more maintenance doses to said patient.
79. The use according to claim 78, further comprising administering a second loading dose to said patient.
80. The use according to claim 79, wherein said second loading dose is suitable for being administered 12 hours after the initial loading dose.
81. The use according to claim 78, wherein each of said maintenance doses is suitable for being administered at a level that is less than the loading dose.
82. The use according to claim 81, wherein each of said maintenance doses is suitable for being administered for 2 hours every 12 hours beginning at hour 24.
83. The use according to claim 57, wherein said patient suffers from pulmonary bacterial infection or pulmonary symptomatic exposure to endotoxin, and wherein said composition is suitable for being administered by periodic bolus administration, by continuous metered inhalation, or by a combination thereof.

84. The use according to claim 79, wherein said composition is suitable for being administered by inhalation as a single dose of 1 μ g-24 mg of the compound of formula (I).
85. The use according to claim 84, wherein said composition is suitable for being administered by inhalation as a single dose of 5-150 μ g of the compound of formula (I).
86. The use according to claim 85, wherein said composition is suitable for being administered by inhalation as a single dose of 10-100 μ g of the compound of formula (I).
87. The use according to claim 84, wherein said composition is suitable for being administered by inhalation as a single dose of 5 mg of the compound of formula (I).
88. The use according to claim 83, wherein said composition is suitable for being administered by inhalation from 1 to 4 times per day.
89. The use according to claim 88, wherein said composition is suitable for being administered by inhalation from 2 to 3 times per day.
90. The use according to claim 88, wherein said composition is suitable for being administered by inhalation once per day.
91. The use according to claim 84, wherein said composition is nebulized or aerosolized.