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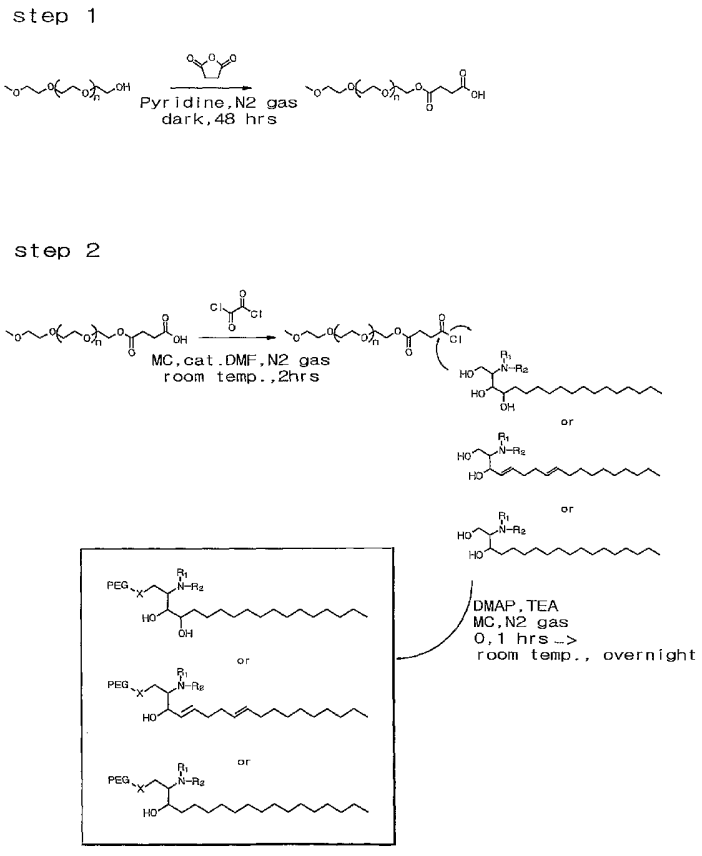
(54) Title: SPHINGOLIPID DERIVATIVES MODIFIED BY POLYETHYLENE GLYCOL AND COMPOSITION CONTAINING THE SAME



(57) Abstract: The present invention relates to sphingolipid derivatives modified by polyethylene glycol which have superior stability and solubility to conventional sphingolipid derivatives and remarkably reduced toxicity, wherein the sphingolipid is selected from the group consisting of phytosphingosine, sphiganine and sphingadiene. The sphingolipid derivatives are effective in stabilizing other active ingredients in the form of liposome compositions, etc.

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SPHINGOLIPID DERIVATIVES MODIFIED BY POLYETHYLENE GLYCOL AND COMPOSITION CONTAINING THE SAME

TECHNICAL FIELD

The present invention relates to sphingolipid derivatives, and more particularly to sphingolipid derivatives modified by polyethylene glycol.

BACKGROUND ART

Until now, many novel drugs have been developed from various origins including low-molecular weight substances, macromolecules, proteins, peptides and so on. These drugs are introduced into the body through a suitable route such as oral administration, subcutaneous injection, intravenous injection, topical application or the like, and circulate in the body along the bloodstream. The circulating drugs arrive at specific target sites and exert their efficacy, and are thereafter degraded in the liver and filtered in the kidneys, eventually being released from the body. However, in the course of circulation, some drugs may cause toxicity even at normal sites other than affected sites. Alternatively, some drugs may be entrapped by the immune system and degraded before sufficiently exerting their efficacy at target sites. Although a drug has excellent efficacy, it cannot effectively exert its efficacy because of side effects, for example, poor stability of the drug itself and occurrence of immune response caused by the immune system. In practice, it has been revealed that toxicity and ineffective delivery to appropriate drug delivery systems account for 80% of failures in clinical tests of drugs developed hitherto. In this connection, extensive studies for improving the stability, reducing immune response, preventing the degradation and extending retention time of drugs in the body are being undertaken.

Polyethylene glycol is a substance represented by the chemical formula HO-$(CH_2CH_2O)_nCH_2CH_2$-OH, and is a significant component of foods and cosmetics. Since polyethylene glycol can delay the degradation of active ingredients, it is widely used in drug prescriptions. In light of the fact that small-sized drugs are likely to be rapidly degraded in the body, the selection of suitable kinds and sizes of polyethylene glycol enables control over the activation time of drugs. Particularly, since polyethylene glycol is a non-toxic and highly soluble polymer, exhibits no antigenicity in humans and is readily degraded in the body, it is suitable for use in foods, cosmetics and drugs. Polyethylene glycol contains hydroxyl groups at both ends of its hydrocarbon chain. Monomethoxy polyethylene glycol (CH_3O-$(CH_2CH_2O)_nCH_2CH_2$-OH) wherein one end is inactivated (blocked) by a methoxy group is widely used for PEGylation. The monomethoxy polyethylene glycol has a maximum molecular weight of 30,000 daltons. In order to facilitate binding of monomethoxy polyethylene glycol to drugs, hydroxyl group at the other end, i.e., not linked by a methoxy group, is activated by numerous chemicals.

Pegylation, a reaction for binding polyethylene glycol to various kinds of drugs, has been applied to drug development since the 1970's. In particular, when applying pegylation not only to interferon-alpha for treating chronic hepatitis, but also to doxorubicin, adenosine deaminase, interleukin-2, tumor necrosis factor-alpha and human growth hormone for treating cancers or AIDS-related kaposi's sarcoma, better results are obtained.

Pegylation can serve numerous effects. First, pegylation can assist in improving stability and solubility of drugs. When hydrophilic polyethylene glycol is linked to a drug, the long chain of the polyethylene glycol surrounds the drug, thus improving the solubility of the drug and stably maintaining the drug in water. Second,

since polyethylene glycol exhibits no antigenicity in humans, it can reduce the occurrence of immune response and can prevent the degradation of drugs by proteins. Third, due to the high molecular weight of polyethylene glycol, a time taken in filtering of drugs in the kidneys is delayed and eventually the retention time of the drugs in the body is extended. That is, since polyethylene glycol-linked drugs exhibit EPR effect (enhanced permeability and retention effect), the drugs become specific to cancer cells (particularly, liver cancer cells). Considering the finding that blood vessels of cancer cells are weaker than those of normal cells, studies on the selective delivery of drugs to cancer cells through size-controlled liposomes without negatively affecting normal cells are being actively undertaken. Fourth, since polyethylene glycol extends the retention time of drugs in the body, the injection intervals of the drugs are lengthened and thus inconvenience to patients can be removed. Fifth, since polyethylene glycol allows drugs to stay in the body for an extended time, optimum distribution of the drugs in the body can be induced. Sixth, since polyethylene glycol can reduce the toxicity of drugs in the body, the dose of the drugs can be freely increased to amounts sufficient to reach target internal organs.

Liposomes are vesicles comprised of concentrically ordered lipid bilayers which can encapsulate a variety of bioactive materials. Liposomes form when lipids, molecules which typically comprise a polar head group attached to one or two long chain aliphatic tails, such as phospholipids, are exposed to water. That is, when liposomes are surrounded by external water, they aggregate to form a structure in which only the polar head groups are exposed to the external water to form an external shell inside which the non-polar (hydrophobic) tails are sequestered. Liposomes can entrap various bioactive materials and drugs therein, and can deliver the materials and drugs to specific target cells and tissues. The materials and drugs entrapped in the liposomes are protected

from interactions with serum factors which may chemically degrade the drug. The controlled size of the liposomes and the change in the structure of liposome-constituting membranes also affect the access of the liposomes to certain sites in the body. The application of polyethylene glycol-lipid polymers or gangliosides to liposomes can prevent the liposome from being absorbed by the reticuloendothelial system. Liposomes can exist in a variety of structures. Typical classes of liposome structures include small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs) and multilamellar vesicles (MLVs).

Constituent materials of liposome essentially include a phospholipid and cholesterol as a stabilizer. Many lipid derivatives have been developed to form novel liposome structures. Of these, polyethylene glycol-derivatized lipids are described in U.S. Pat. No. 5,013,556, 1991 issued to Woodle. The polyethylene glycol-lipid polymers generally have a structure in which polyethylene glycol is linked to the polar head group of diacylglycerophospholipid. Since these phospholipids usually contain two fatty acid groups bonded to the 1- and 2- position of glycerol by ester linkages, they are susceptible to cleavage under acidic or basic conditions. The cleavage of fatty acid groups under acidic or basic conditions leads to the formation of lysophospholipid and glycerophosphate, which easily escape from the bilayer structure of the liposomes, thus weakening the rigidity of the liposomes. Since such weakened rigidity causes significant leakage of drugs present inside the liposomes, many problems occur during use, and the benefits of the polyethylene glycol-lipid polymers cannot be further attained. Lipid stability is especially important in the case that a hydrogen ion concentration (pH) gradient is used to entrap bioactive agents in liposomes. Therefore, it is desirable to develop lipids or polyethylene glycol-lipid polymers that are less susceptible to hydrolysis.

Sphingolipids are a generic name for composite lipids comprising a ceramide and a polar head group modified to C1-OH of the ceramide, the ceramide including a sphingoid long-chain base, such as sphingomyelin or glycosphingolipid, consisting of 18 carbon atoms, as a backbone, and one or two fatty acids amide-bonded to its 2-amino group.

Sphingolipids are constituent components essential for forming cell membranes of higher animals, and performs critical regulatory roles in bioactivity. Thus, there have been a variety of studies on the functions of sphingolipids. Since sphingolipid was named 'sphinx like' by Thudichum in 1884, it has drawn attention as a second messenger in the signal transduction pathway. Recent research has revealed that the signal transduction pathway of sphingolipids is accomplished through the sphingomyelin cycle, and ceramides produced by external signals activate the signal transduction system which participates in cell growth, differentiation, aging and death of cells. It is known that sphingomyelinase activated by various ligands such as TNF-alpha and gamma-IFN degrades sphingomyelin into a ceramide, which is then transformed into sphingosine and sphingosine-1-phosphate. Ceramide, sphingosine and sphingosine-1-phosphate play roles as second messengers mediating the functions of various signal transducers.

In particular, it is believed that sphingosine and sphingosine-1-phosphate perform functions contrary to each other, and play a critical roll in life phenomena, e.g., induction of cell growth or death. Sphingosine mainly induces cell apoptosis, whereas sphingosine-1-phosphate induces cell growth and differentiation. Based on the effects of sphingosine on apoptosis and cell mobility, there have been many technological advances in developing anticancer agents.

Naturally occurring sphingolipids largely have basic backbones such as sphinganine, sphingosine and phytosphingosine. Sphingolipids based on sphingosine

as a basic backbone are predominantly found in animals including humans, while sphingolipids based on phytosphingosine as a basic backbone are mainly found in plants and fungi. Recent research on sphingolipids has mainly focused on the sphingolipids having a sphingosine backbone. Since phytosphingosine is predominantly present in the skin, it is partially used only in the cosmetic industries. However, recent research results show that phytosphingosine exhibits almost the same effects as sphingosine, and in some cases, exhibits excellent biological activities compared to sphingosine. There is, thus, a growing interest toward phytosphingosine. Further, since sphingolipids having a sphingosine backbone are obtained by extracting animal internal organs or eggs, considerable costs are required to obtain their pure forms. However, since phytosphingosine derivatives are mass-produced in their pure forms by yeast fermentation, they can be used not only as functional materials but also in various formulations of cosmetics and medicines.

The present inventors have conducted much research with the aim of developing various sphingolipid derivatives having excellent bioactivity and to extend the applications of sphingolipid derivatives. Functions of sphingolipids depend on the structure of the sphingolipids. Ceramides have been generally utilized as main raw materials of humectants worldwide. Since it has been found that ceramides are implicated in skin diseases such as atopic dermatitis, psoriasis and acne, great efforts have been undertaken to treat the skin diseases using ceramides (Arch. Dermatol. Res. 1997, 289-). However, since ceramides have a difficulty in their use at a high concentration in cosmetics due to their poor stability, many attempts have been made to improve the solubility of ceramides.

On the other hand, it is known that phytosphingosine long chain base not only exhibits stronger antibacterial activity against harmful bacteria, including *Staphylococcus*

aureus, than conventional antibiotics and inhibitory effects against protein kinase C and phospholipase D, but also exhibits various bioactivities such as apoptosis. However, the problems of cytotoxicity and poor solubility of the phytosphingosine long chain base remain unsolved. The present inventors have earnestly and intensively conducted research to solve the above-mentioned problems of sphingolipids, and as a result, found that polyethylene glycol-sphingosine derivatives can solve cytotoxicity and poor solubility of sphingolipids for use in various applications.

Ceramides having improved solubility can be used for preparing not only general cosmetic and medicinal formulations, but also liposome, lamellar and liquid-crystal formulations for the purpose of improving the stability of the formulations. It is also expected that phytosphingosine long chain base derivatives having reduced toxicity and improved solubility can be used in large quantities in cosmetic and pharmaceutical applications, and their effects are thus considerably exerted.

In connection with the present invention, synthesis of polyethylene glycol-ceramide derivatives and their applications to liposomes are described in U.S. Pat. No. 5,820,873 (1995) to Lewis S. L. *et al.* However, the polyethylene glycol-ceramide derivatives are those obtained by simply linking polyethylene glycol to ceramides.

No technology concerning the binding of polyethylene glycol to sphinganine and sphingadiene, including phytosphingosine known for its usefulness, has been reported until now.

DISCLOSURE OF THE INVENTION

Therefore, the present invention has been made in view of the above problems, and it is an object of the present invention to provide highly stable and soluble sphingolipid derivatives without any degradation by proteins due to reduced immune

response wherein the sphingolipid is selected from the group consisting of phytosphingosine, sphinganine and sphingadiene. The sphingolipid derivatives have been recently known for their usefulness.

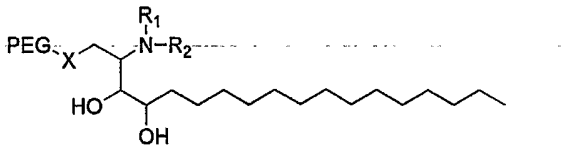
It is another object of the present invention to provide sphingolipid derivatives which have extended retention time of active ingredients in the body so as to induce specificity to cancer cells (particularly, liver cancer cells).

It is another object of the present invention to provide sphingolipid derivatives which have reduced toxicity in the body so as to freely increase the dose of drugs to amounts sufficient to reach target internal organs.

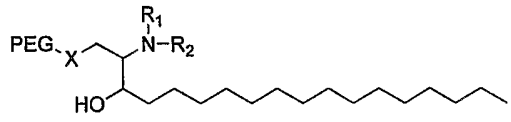
It is yet another object of the present invention to provide sphingolipid derivatives which are effective in stabilizing and dissolving other active ingredients, can extend the duration of efficacy and can be used as components of liposome compositions for toxicity reduction.

In order to accomplish the above objects of the present invention, there are provided sphingolipid derivatives modified by polyethylene glycol wherein the sphingolipid is selected from the group consisting of phytosphingosine, sphinganine and sphingadiene, and the polyethylene glycol has an average molecular weight of 550~10,000, the respective sphingolipid derivatives being represented by Formulae 1~3 below:

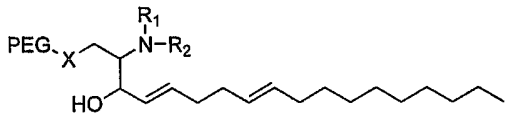
Formula 1



Formula 2



Formula 3



wherein R_1 and R_2 are each independently a hydrogen, a $C_{1\sim40}$ alkyl group, a $C_{1\sim40}$ alkenyl group, a $C_{1\sim40}$ alkynyl group, an acyl group represented by $-COR_3$ (where R_3 is an alkyl, alkenyl, alkynyl or aryl group), or aryl group; and

X represents a linker used to bind the polyethylene glycol to the sphingolipids, and is $-NR_4-$ (where R_4 is a hydrogen, a $C_{1\sim6}$ alkyl group, an acyl group or an aryl group), -O-, -S- or X_1-alk-X_2 (where X_1 and X_2 are each independently an amino, amido, carboxyl, carbamate, carbonyl, urea or phosphoro group, and alk is a $C_{1\sim6}$ alkylene group.

Of these, preferred sphingolipid derivatives include those wherein R_1 and R_2 are each independently a hydrogen, an alkyl or acyl group, X is succinate, and the polyethylene glycol has a molecular weight of 750~5,000 and its end is substituted with a methoxy group.

In accordance with another aspect of the present invention, there are provided an anticancer composition, an antibacterial composition, an anti-inflammatory composition, a composition for signal transduction activation and a composition for treating skin diseases, each comprising at least one compound of the sphingolipid derivatives as an active ingredient.

In accordance with yet another aspect of the present invention, there is provided a liposome composition comprising at least one compound of the polyethylene glycol-modified phytosphingosine derivative, the polyethylene glycol-modified sphinganine derivative and the polyethylene glycol-modified sphingadiene derivative. The liposome composition may further comprise DOPE, DODAC, DSPC, SM or Chol. The sphingolipid derivatives of the present invention can be used for preparing not only liposome formulations, but also lamellar and liquid-crystal formulations.

The present invention is characterized in that the liposome composition further comprises at least one bioactive material entrapped therein. The term 'bioactive material' used herein includes anticancer agents, antibiotics, immuno-modulating agents, anti-inflammatory agents, vaccines and drugs acting on the central nervous system. Specifically, the bioactive material is a gene, an oligonucleotide, a protein, a peptide, a hormone or a vitamin.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

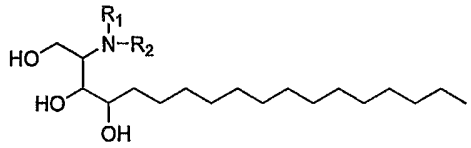
Fig. 1 is a reaction scheme showing a process for the preparation of polyethylene glycol-modified sphingolipid derivatives according to the present invention; and

Fig. 2 is a reaction scheme showing a process for the preparation of a polyethylene glycol-modified phytosphingosine derivative according to an embodiment of the present invention.

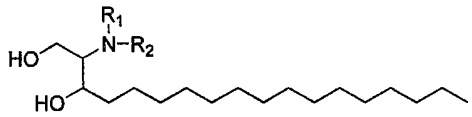
BEST MODE FOR CARRYING OUT THE INVENTION

Sphingolipid derivatives of the present invention can be prepared in accordance with the following procedure. First, sphingolipids are reacted with polyethylene glycol previously activated in a suitable solvent in the presence of a catalyst, to prepare corresponding sphingolipid derivatives. Specifically, the sphingolipids used to prepare the sphingolipid derivatives of Formulae 1 to 3 above are represented by Formulae 4 to 6 below:

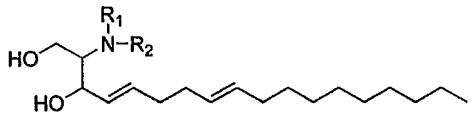
Formula 4



Formula 5



Formula 6



Hereinafter, a method for preparing the sphingolipid derivatives of the present invention will be explained in more detail with reference to the accompanying drawings. As depicted in Fig. 1, first, polyethylene glycol is activated. Succinic anhydride is added to the polyethylene glycol in an organic solvent to transform the alcohol group into a carboxyl group. Thereafter, the carboxyl group is activated into a chloride form using

oxalylchloride, after which the chloride form is added dropwise to each sphingolipid in the presence of a catalyst and reacted at room temperature for 24 hours.

The compounds of Formulae 1 to 3 are extracted with chloroform or a mixed solvent of chloroform/methanol, and then purified by adsorption chromatography (adsorbent: silica gel).

On the other hand, the present invention provides liposomes or carriers formed using the polyethylene glycol-sphingolipid derivatives. Generally, liposomes can be formed from representative examples of the compounds according to the present invention. Another lipid form such as DOPE, DODAC, DSPC, SM or Chol can be further added to form the liposomes.

The content of the compounds according to the present invention is preferably in the range of 0.01~90 mole%, based on the total liposome-constituent components. The content may vary, e.g., 5~30 mole%, 0.01~60 mole%, 0.5~5 mole%, etc., according to drugs to be delivered by the liposomes. When the content is less than 0.01 mole%, no effect is accomplished. When the content exceeds 90 mole%, the stability of the composition is damaged.

An anticancer composition, an antibacterial composition, an anti-inflammatory composition, a composition for signal transduction activation or a composition for treating skin diseases which comprises at least one compound of the sphingolipid derivatives as an active ingredient, may be administered through a suitable route such as oral, parenteral, rectal, vaginal, local, transdermal, intravenous, intramuscular, intraperitoneal or subcutaneous route. The amount of the composition to be administered will, of course, be dependent on the subject to be treated, the kind and severity of the particular affliction or condition, the route of administration, the judgment of the prescribing physician, etc. Based on these factors, the dose may be appropriately

determined by a person skilled in the art. The dose may commonly range between about 0.001mg/kg/day and 2,000 mg/kg/day, and preferably between about 0.5mg/kg/day and 2.5 mg/kg/day.

The sphingolipid derivatives of the present invention may be formulated with pharmaceutically acceptable carriers to prepare pharmaceutical compositions. General methods for preparing pharmaceutical compositions and typical carriers used to prepare the compositions are described in Remington's Pharmaceutical Sciences, latest edition, by E. W. Martin, Merck Publ. Co., Easton, PA. The compounds of the present invention can be administered together with anticancer agents. Further, the compositions of the present invention can be administered along with other therapeutic compositions through appropriate operations. For example, the compositions of the present invention can be administered, in conjunction with a treatment such as surgical resection, radiotherapy or chemotherapy.

Depending on an intended administration mode, the pharmaceutical compositions may be solid, semi-solid or liquid form. The formulations include dosage forms such as tablets, pills, capsules, suppositories, sachets, granules, powders, creams, lotions, ointments, plasters, liquid solutions, suspensions, dispersions, emulsions and syrups, but are not limited thereto. The active ingredients may be entrapped by liposomes, particulates and microcapsules.

Examples of commonly used non-toxic carriers include, but are not limited to, mannitol, lactose, starch, magnesium stearate, sodium saccharine, talc, cellulose, glucose, sucrose, dextrose, glycerol, magnesium carbonate, triglyceride, oils, solvents, sterile water and isotonic saline solution. Solid formulations such as tablets, pills and granules may be coated for convenient use. Compositions for intravenous administration are typically solutions in sterile isotonic aqueous buffers, and comprise a local anesthetic for

relieving pain at injection sites. If desired, drugs may comprise a small amount of a non-toxic auxiliary substance such as wetting agents, emulsifier, pH buffer, etc. Examples of the auxiliary substance include, but are not limited to, sodium acetate, sorbitan monolaurate, triethanolamine and triethanolamine oleate. The compositions of the present invention may further comprise an additive selected from stabilizers, antioxidants, binders, colorants, flavors, preservatives and thickening agents.

In order to evaluate the physicochemical properties of the sphingolipid derivatives according to the present invention, the present inventors tested the solubility and cytotoxicity of the sphingolipid derivatives. As a result of a series of experiments, it was observed that the sphingolipid derivatives exhibit considerably improved solubility not only in organic solvents but also in aqueous solutions, and confirmed that insolubility, a main problem in the use of lipids, can be solved. In addition, cytotoxicity experiments on skin cancer cells (HacaT) revealed that the sphingolipid derivatives of the present invention exhibit remarkably reduced cytotoxycity, compared to sphingolipids not modified by polyethylene glycol.

The present invention will now be described in more detail with reference to the following examples and experimental examples. However, these examples and experimental examples are given for the purpose of illustration and are not to be construed as limiting the scope of the invention.

Example

<Example 1: Preparation of sphingolipid derivatives according to the present invention>

A reaction scheme showing a process for the preparation of polyethylene glycol-modified sphingolipid derivatives according to the present invention, is depicted

in Fig. 2. As depicted in Fig. 2, 5.0g (0.0067 mole) of methoxypolyethylene glycol was dissolved in pyridine under a nitrogen atmosphere, and then 6.67g (0.067 mole) of succinic anhydride was slowly added thereto. The mixture was reacted at room temperature for 48 hours in a state in which light was completely blocked to yield methoxypolyethylene glycol carboxylic acid. After the reaction was completed, hydrochloric acid was added to adjust the reaction mixture to pH 2. At this time, the pyridine was transformed into a salt form. The salt form was removed by extraction using chloroform and water. Unreacted succinic anhydride was removed by extraction using water. The methoxypolyethylene glycol carboxylic acid was activated by oxalylchloride, and then allowed to react with respective sphingolipids to prepare the compounds of Formulae 1 to 3. Specifically, 1.0g (0.001176 mole) of methoxypolyethylene glycol carboxylic acid was dissolved in dichloromethane under a nitrogen atmosphere, and then 0.299g (0.002352 mole) of oxalylchloride was added dropwise thereto in the presence of dimethylformamide. The reaction was continued at room temperature for 2 hours. After the reaction was completed, unreacted oxalylchloride was removed by stripping using chloroform. A sphingolipid was bonded to the reaction product without further purification. As the sphingolipid, ceramide 3 in which a fatty acid having 18 carbon atoms is bonded to the amine group of phytosphingosine, was used. 0.641g (0.001 mole) of the ceramide 3 was dissolved in dichloromethane, and then small amounts of dimethylaminopyridine and triethylamine as catalysts were added thereto. To the resulting solution, chlorinated methoxypolyethylene glycol dissolved in a small amount of dichloromethane at 0°C was slowly added dropwise. After 1 hour, the temperature was raised to room temperature. The reaction was sufficiently continued for 24 hours. The reaction mixture was extracted with distilled water to stop the reaction, and purified by adsorption

chromatography (adsorbent: silica gel) to prepare a phytosphingosine derivative of Formula 1. The existence of succinic acid introduced into the methoxypolyethylene glycol by the reaction with the succinic anhydride was identified through ^{1}H-NMR analysis (2.6 ppm, t, 4H). In addition, the preparation of the phytosphingosine derivative in which the methoxypolyethylene glycol was bonded to the ceramide 3 according to the present invention was confirmed by the existence of the following ^{1}H-NMR peaks: methylene (1.2 ppm, m, 48H) and methyl (0.9 ppm, t, 6H) of the sphingolipid, and ethoxy (3.6 ppm, m, 64H) and methoxy (3.3 ppm, s, 3H) of the methoxypolyethylene glycol. The molecular weight of the phytosphingosine derivative was measured using MALDI-MASS (calculated value: 1414.86, found value: 1414.99). Fig. 2 shows steps for the preparation of the phytosphingosine derivative.

<Example 2: Solubility test>

In order to evaluate the water-solubility of methoxypolyethylene glycol-ceramide 3 prepared in Example 1 as a sphingolipid derivative, the present inventors measured the water-solubility of ceramide 3 as a control group. As a result, it was observed that only approximately 0.70% of the ceramide 3 was dissolved in distilled water and undissolved portions were settled to the bottom. In contrast, a maximum of 2.0% of the sphingolipid derivative was dissolved in distilled water and homogeneously dispersed in the solution.

<Example 3: Cytotoxicity test>

The cytotoxicity of the sphingolipid derivatives according to the present invention was evaluated by MTT assay. As a cell line for the experiment, HaCaT (Human keratinocyte cell line) was used. The polyethylene glycol-modified

phytosphingosine derivative was used as a test group, and phytosphingosine was used as a control group. The phytosphingosine-polyethylene glycol derivative and the phytosphingosine were separately dissolved in DMSO. The HaCaT cells were inoculated on a 96-well multi-plate in an amount of 1 x 10^4 cells/well, and then incubated in a 5% CO_2 incubator at 37°C for 24 hours. After incubation, the respective samples dissolved in serum-free RPMI were treated at various concentrations and further incubated for 24 hours. Thereafter, MTT at a final concentration of 0.5mg/ml was added to each well, and then incubated for 3 hours. The absorbance of the wells was read at 570nm. The results are shown in Table 1 below. The data are expressed as survival rate (%).

Table 1

Concentration (μM)	Phytosphingosine (Control group)	Polyethylene glycol-modified phytosphingosine derivative (Test group)
1	98	98
2	46	87
3	25	56
4	10	38
5	4	22
10	0	10

As can be seen from the data shown in Table 1, the polyethylene glycol-phytosphingosine derivative of the present invention exhibited lower toxicity than phytosphingosine.

<Example 4: Formation of liposome>

The present inventors formed liposomes using the sphingolipid derivatives of the present invention.

A mixture of distearoylphosphatidylcholine (DSPC) and cholesterol (60:40 by molar ratio) was used as a control composition, and a mixture of distearoylphosphaticylcholine (DSPC), cholesterol and a polyethylene glycol-sphingolipid derivative (55:40:5 by molar ratio) was used as a test composition. The polyethylene glycol used herein had a molecular weight of 750, and ceramide 3 was used as the sphingolipid (having a phytosphingosine backbone). Each lipid was dissolved in chloroform, and then dried under a stream of nitrogen gas to obtain dried lipid films. After the dried lipid films were placed under vacuum for 2 hours, a buffer containing HEPE was added to hydrate the lipid films. The resulting lipid films were cut to obtain liposomes having an average size of 100nm. The liposomes thus obtained were diluted and injected into rats (50μM/kg). After blood samples were obtained from the rats with the lapse of time, the content of Taxol entrapped in the liposomes was measured and then compared to the retention time in the body. The results are shown in Table 2 below. The values represent the amount of the active ingredient (μM/100μl plasma) remaining in the body.

Table 2

Time	Test liposomes	Control liposomes
0	0.2	0.2
After 5 hours	0.14	0.07
After 12 hours	0.08	0.03
After 24 hours	0.02	0

As can be seen from the data shown in Table 2, the liposomes containing the

polyethylene glycol-sphingolipid derivative of the present invention stayed in the body for a relatively extended time, compared to the control liposomes.

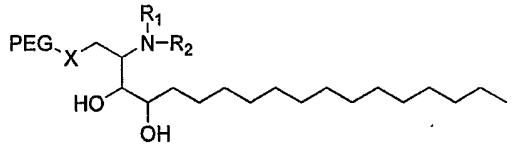
INDUSTRIAL APPLICABILITY

As apparent from the above description, since the polyethylene glycol-modified sphingolipid derivatives of the present invention can maintain the bioactivity of the sphingolipid derivatives, they can be used as effective ingredients not only of anticancer compositions, antibacterial compositions and anti-inflammatory compositions, but also of cosmetics and medicines targeting various signal transduction systems. In addition, the polyethylene glycol-modified sphingolipid derivatives of the present invention are excellent in terms of improved stability and solubility, extended duration of efficacy in the body, and remarkably reduced toxicity, compared to conventional sphingolipid derivatives. In addition, the polyethylene glycol-modified sphingolipid derivatives of the present invention are effective in formulation stabilization of other active ingredients, because fatty acid chains of the lipids are hydrolyzed. Since the hydrolysis prevents entrapped materials from leaking outside, the polyethylene glycol-modified sphingolipid derivatives can be used as main raw materials for forming liposomes or stabilizers of liposomes. Since the liposomes thus formed can be safely introduced into the body through a suitable route such as oral administration, injection or topical application, they are useful in a wide range of applications.

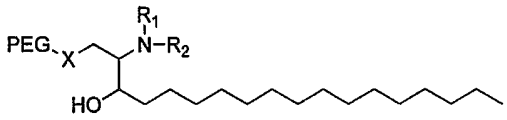
Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

WHAT IS CLAIMED IS:

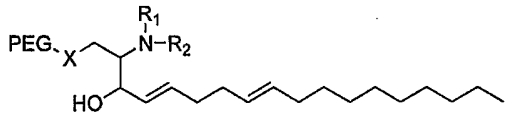
1. A polyethylene glycol-modified sphingolipid derivative selected from the group consisting of a polyethylene glycol-modified phytosphingosine derivative, a polyethylene glycol-modified sphinganine derivative and a polyethylene glycol-modified sphingadiene derivative, represented by Formulae 1~3 below, respectively:



(1)



(2)



(3)

wherein R_1 and R_2 are each independently a hydrogen, an alkyl group, an alkenyl group, an alkynyl group, an acyl group or an aryl group; and

X represents a linker used to bind the polyethylene glycol to the sphingolipids, and is $-NR_4-$ (where R_4 is a hydrogen, a $C_{1\sim6}$ alkyl group, an acyl group or an aryl group), -O-, -S- or X_1-alk-X_2 (where X_1 and X_2 are each independently an amino, amido, carboxyl, carbamate, carbonyl, urea or phosphoro group, and alk is a $C_{1\sim6}$ alkylene group.

2. The polyethylene glycol-modified sphingolipid derivative according to claim 1, wherein R_1 and R_2 are each independently an acyl group represented by $-COR_3$ (where R_3 is an alkyl, alkenyl, alkynyl or aryl group).

3. The polyethylene glycol-modified sphingolipid derivative according to claim 1, wherein the polyethylene glycol has an average molecular weight of 550~10,000.

4. The polyethylene glycol-modified sphingolipid derivative according to claim 1, wherein R_1 and R_2 are each independently a $C_{1\sim40}$ alkyl, $C_{1\sim40}$ alkenyl or $C_{1\sim40}$ alkynyl group.

5. An antibacterial composition comprising the polyethylene glycol-modified sphingolipid derivative according to any one of claims 1 to 4 as an active ingredient.

6. An anti-inflammatory composition comprising the polyethylene glycol-modified sphingolipid derivative according to any one of claims 1 to 4 as an active ingredient.

7. An anticancer composition comprising the polyethylene glycol-modified sphingolipid derivative according to any one of claims 1 to 4 as an active ingredient.

8. A composition for treating skin diseases, comprising the polyethylene glycol-modified sphingolipid derivative according to any one of claims 1 to 4 as an active ingredient.

9. A composition for signal transduction activation, comprising the polyethylene glycol-modified sphingolipid derivative according to any one of claims 1 to 4 as an active ingredient.

10. A liposome composition comprising the polyethylene glycol-modified sphingolipid derivative according to any one of claims 1 to 4 as an active ingredient.

11. The liposome composition according to claim 10, wherein the polyethylene glycol-modified sphingolipid derivative is at least one compound of the polyethylene glycol-modified phytosphingosine derivative, the polyethylene glycol-modified sphinganine derivative and the polyethylene glycol-modified sphingadiene derivative.

12. The liposome composition according to claim 11, further comprising DOPE, DODAC, DSPC, SM or Chol.

13. The liposome composition according to claim 11, further comprising at least one bioactive material entrapped therein.

14. The liposome composition according to claim 13, wherein the bioactive material is an anticancer agent, an antibiotic, an immuno-modulating agent, an anti-inflammatory agent, a vaccine or a drug acting on the central nervous system.

15. The liposome composition according to claim 14, wherein the bioactive material is a gene, an oligonucleotide, a protein, a peptide, a hormone or a vitamin.

16. The liposome composition according to claim 10, wherein the polyethylene glycol-modified sphingolipid derivative is present in the range of 0.01~90 mole%.

Fig.1

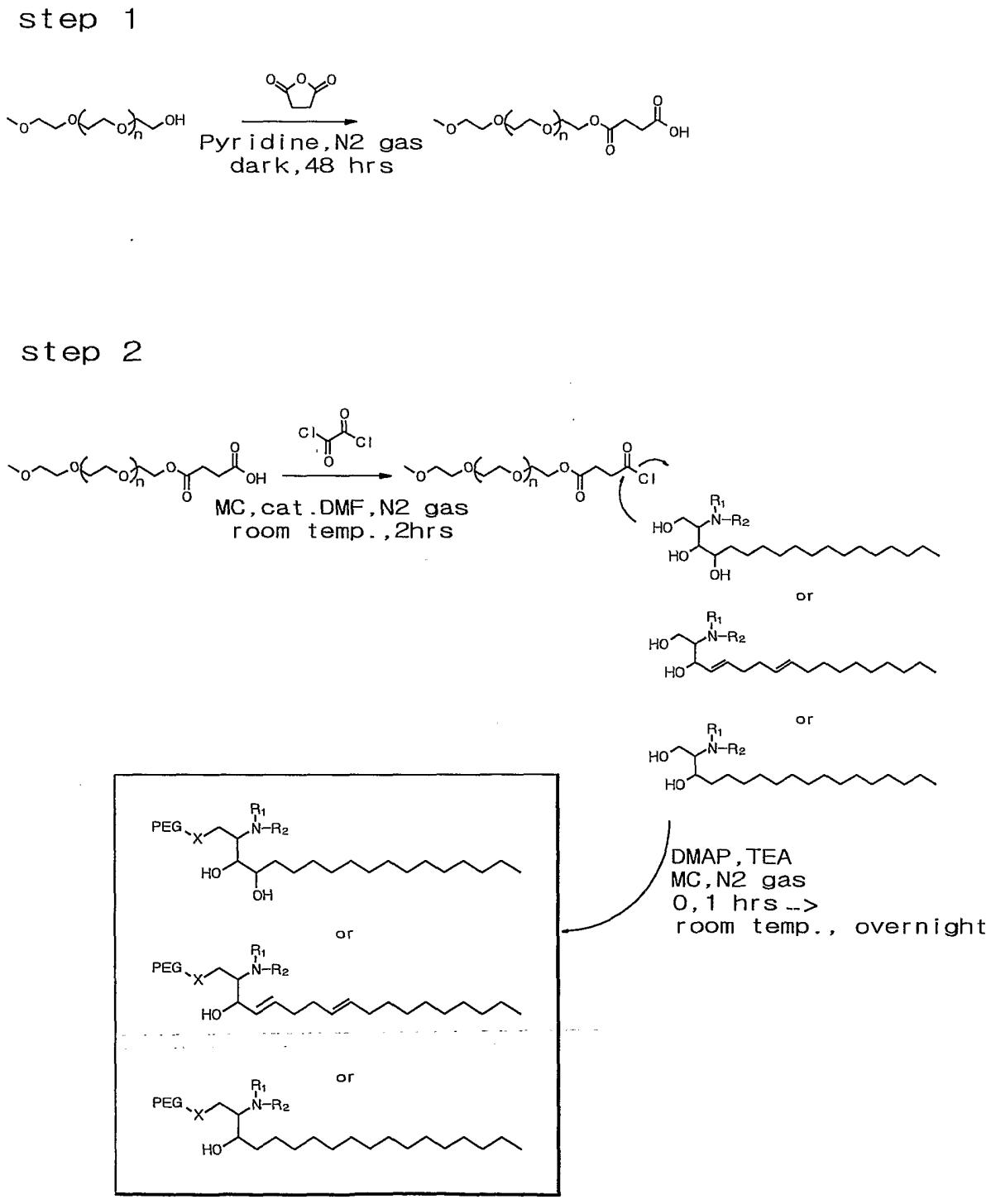
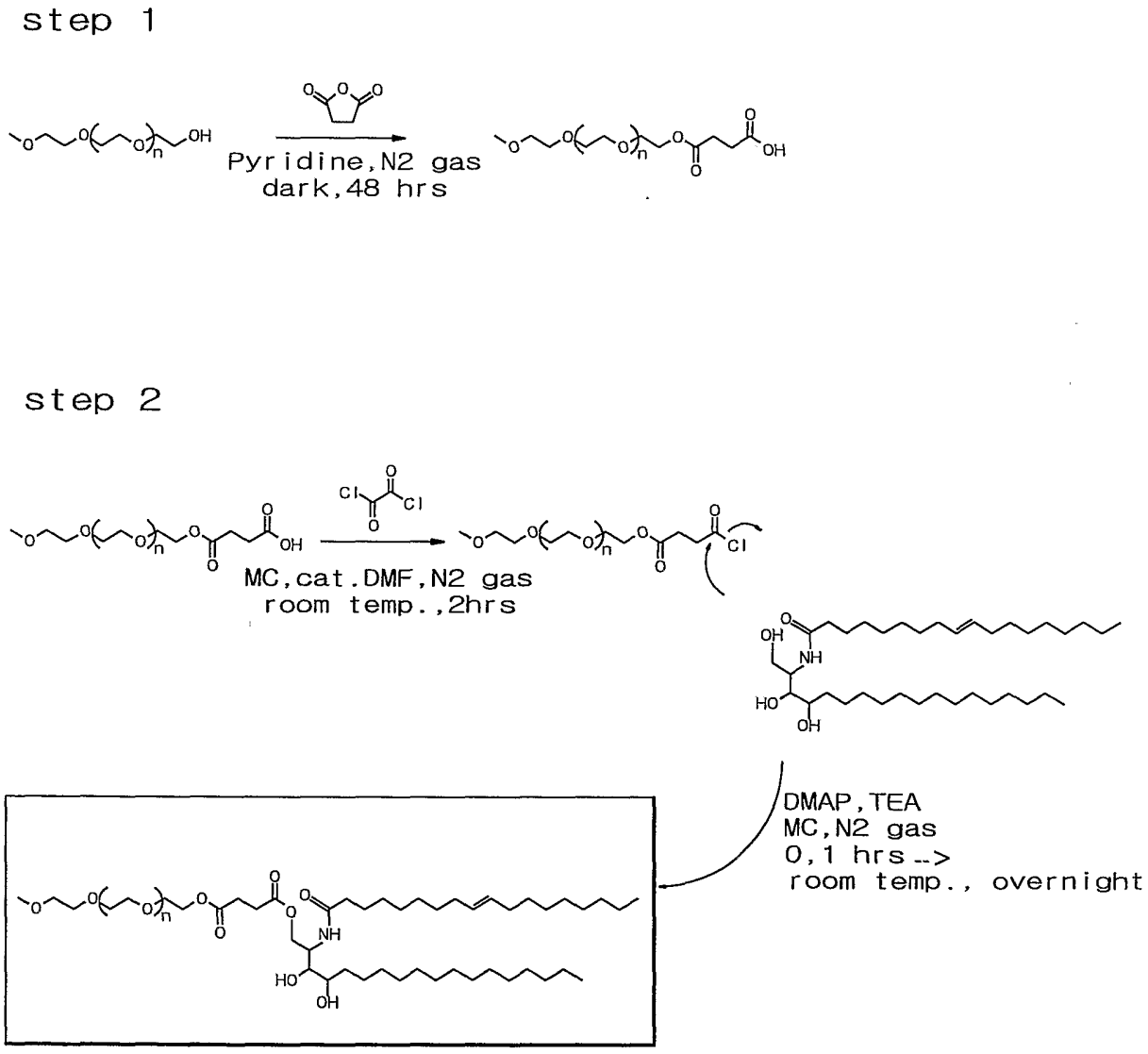

step 1
Pyridine,N2 gas
dark,48 hrs
step 2
MC,cat.DMF,N2 gas
room temp.,2hrs
or
or
DMAP,TEA
MC,N2 gas
0,1 hrs --->
room temp., overnight
or
or

Fig.2


step 1
Pyridine,N2 gas
dark,48 hrs
step 2
MC,cat.DMF,N2 gas
room temp.,2hrs
DMAP,TEA
MC,N2 gas
0,1 hrs -->
room temp., overnight

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2003/002716

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07C 215/02, A61K 31/133

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC7 C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean patents and applications for invention since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN(CA)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5820873 A(UNIV BRITISH COLUMBIA) 13 OCTOBER 1998 see claims	1-16
X	Murray S. Webb et al., "Comparison of different hydrophobic anchors conjugated to poly(ethylene glycol): effects on the pharmacokinetics of liposomal vincristine", Biochimica et Biophysica Acta, 1998, 1372(2), 272-282 see the whole document	1-16
A	US 5225212 A(LIPOSOME TECHNOLOGY INC) 6 JULY 1993 see the whole document	1-16
A	Klibanov A. L. et al., "Activity of amphipathic poly(ethylene glycol) 5000 to prolong the circulation time of liposomes depends on the liposome size and is unfavorable for immunoliposome binding to target", Biochimica et Biophysica Acta, 1991, 1062(2), 142-148 see the whole document	1-16

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 MARCH 2004 (13.03.2004)	15 MARCH 2004 (15.03.2004)

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